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EXPERIMENTAL TERATOGENESIS WITH BENZYL ALCOHOL

Preliminary Report

P. K. DURAISWAMI

The Robert W. Johnson Laboratory of the Louise C. Rowles Research Fund, The Children's Hospital School, Baltimore, Maryland

Received for publication December 15, 1953

The investigations on the teratogenic potentialities of benzyl alcohol were undertaken as a result of an incidental observation made on control embryos, employed in connection with our previous experiments employing cortisone acetate (1, 2). As the saline suspensions of cortisone acetate contained 1.5 per cent benzyl alcohol as a preservative, one-half of the control eggs had been injected with 0.05 to 0.1 cc. of a saline suspension of 1.5 per cent benzyl alcohol and the other half had been injected with similar volumes of saline alone. The embryos treated with 0.05 to 0.1 cc. of 1.5 per cent benzyl alcohol did not show any obvious abnormalities. However, it was decided to investigate the effects, if any, of injections of small doses of *undiluted* benzyl alcohol into the yolk, with the result that a significantly high proportion of the embryos treated with 0.01 to 0.02 cc. of *undiluted* benzyl alcohol exhibited meningoceles, limb deformities, beak defects such as, arched upper beaks, localized blebs and generalized edema. We, therefore, started systematic investigations on the lines previously employed with insulin (1, 2, 3). Injections of different doses of benzyl alcohol were made into the yolk, either before the incubation of the eggs or after the beginning of their incubation, at varying intervals. This is a preliminary report. It is proposed to continue the investigations and to publish later a more detailed report.

Féré (5, 6) induced cyclopia, anophthalmia and defects in the nervous system with certain alcohols such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl and amyl alcohols. Féré injected 0.05 cc. of each of the above-mentioned alcohols into the *albumen* of chicken eggs in order to investigate its toxicity and teratogenic properties. He observed that both the toxicity and "teratogenic power" increased progressively with propyl, butyl and amyl alcohols. However, methyl alcohol was found to be more toxic than ethyl alcohol. The developmental anomalies observed included cyclopia, anophthalmia and "atrophy of the head with torsion". It is interesting to note that we have not observed so far these defects with injections of 0.01 to 0.02 cc. of benzyl alcohol into the *yolk*. From his experimental observations Féré also concluded that the iso-alcohols were more powerful as teratogenic agents than the corresponding normal alcohols.

Stockard (8) subjected the eggs of fish, *Fundulus heteroclitus*, during the

early stages of development, to the action of weak solutions of ethyl alcohol and observed certain abnormalities in the central nervous system, the eyes and the ears of the treated embryos. The ophthalmic defects which occurred in 98 per cent of the treated embryos included anophthalmia, cyclopia and poorly differentiated retinae. Stockard concluded from his experimental results that ethyl alcohol suppressed the growth and differentiation of the brain, spinal cord, eyes and ears. Other workers (7, 9) have confirmed these results with ethyl alcohol in different animals. However, we have not so far come across any reference in the literature to the teratogenic properties of *benzyl alcohol*.

MATERIAL AND METHODS

These experiments were conducted with eggs from New Hampshire red chickens which were known to be free from genetic abnormalities as well as nutritional deficiencies. The methods employed for incubation of the eggs and for conducting histological and radiological investigations on chick embryos were, for all practical purposes, the same as described previously (1, 2, 3).

OBSERVATIONS

This preliminary report deals with meningoceles, limb deformities, beak defects, such as arched upper beaks, localized blebs and generalized edema

TABLE I

Survivors of the Eleventh Day of Incubation Showing Various Defects Induced by Benzyl Alcohol*

TIME OF INJECTION IN RELATION TO INCUBATION	DOSE OF BENZYL ALCOHOL (CC)	NO. OF EMBRYOS SURVIVING THE ELEVENTH DAY OF INCUBATION	MENINGOCELES	LIMB DEFECTS	BEAK DEFECTS	LOCALISED BLEBS AND/OR GENERALISED EDEMA OF BODY
Before incubation	0.01	72	18	19	8	11
After incubation						
24 hours	0.01	119	32	38	4	16
48 hours	0.01	121	39	51	18	17
72 hours	0.01	146	46	62	22	11
96 hours	0.01	152	22	73	32	15
72 hours	0.02	164	66	82	40	21
96 hours	0.02	172	38	107	46	20
120 hours	0.02	523	21	64	17	15
144 hours	0.02	112	Nil	57	9	8
168 hours	0.02	87	Nil	41	6	3

Controls

Out of a total of 312 controls scrutinized, after injecting 0.02 c.c. of *saline* into the yolk, either before incubation of the eggs, or from the first through the seventh day after the beginning of incubation, only *seven* were found to be deformed as follows: (1) absence of coccyx and lower part of sacrum, (2) anencephaly, short upper beak and exomphalos, (3) malrotation of both limbs, (4) anophthalmia, exomphalos and crossing of the beaks, (5) buphthalima and malrotation of the right limb, (6) microphthalmia and crossing of the beaks and (7) abduction deformities of the toes of both feet.

* Two or more defects may be present in the same embryo.

which have been observed among the survivors of the eleventh day of incubation, after injections of 0.01 to 0.02 cc. of benzyl alcohol into the yolk either before incubation of the eggs or from the first through the seventh day after the beginning of their incubation. The results of our initial experiments with the above doses of benzyl alcohol are presented in Table, I and the induced defects are illustrated.

For purposes of comparison, meningoceles caused by lead nitrate (3) and limb defects induced by insulin (1, 2, 3) have also been illustrated (Figs. 10 and 8). The defects produced by benzyl alcohol have been compared with those caused by other teratogenic agents such as, soluseptasine and thallium nitrate in an article to be published elsewhere (4).

Thus far, few of the embryos treated with 0.01 to 0.02 cc. of benzyl alcohol have hatched alive and it is, therefore, proposed to inject lower doses of the teratogenic substance to enable as many of the treated embryos as possible to hatch. This will give us an opportunity to verify if at least some of them develop scoliosis and other deformities during the first 3 or 4 months after hatching, as has been observed in the case of insulin-treated chickens.

SUMMARY

Injections of 0.01 to 0.02 cc. of benzyl alcohol into the yolks of fertile eggs, either before incubation, or from the first through the seventh day after the beginning of their incubation give rise to meningoceles, limb deformities, beak defects such as, arched upper beaks, localized blebs and generalized edema.

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EXPLANATIONS OF ILLUSTRATIONS

FIG. 1. All the embryos are sixteen days old. From left to right. (1) Normal control. (2) Embryo showing meningoceles and limb deformities induced by 0.01 cc benzyl alcohol injected into the yolk-sac on the third day of incubation. Note the blebs over the left eye and frontal region. (3) Embryo showing meningocele and limb deformities induced by 0.02 cc benzyl alcohol injected tinto the yolk-sac on the fourth day of incubation.

FIG. 2. All the embryos are fourteen days old. From left to right. (1) Normal control. (2) Embryo showing meningocele, limb deformities and generalized oedema caused by 0.02 cc of benzyl alcohol injected into the yolk-sac on the third day of incubation. (3) Embryo showing angulation deformity of the left tibia and a large bleb over the left eye produced by injection of 0.01 cc benzyl alcohol on the fourth day of incubation.

FIG. 3. All the embryos are eighteen days old. From left to right. (1) Normal control. (2), (3), and (4) embryos showing different types of limb deformities induced by increasing doses of benzyl alcohol injected on the fourth day of incubation.

FIG. 1

FIG. 2

FIG. 3

FIG. 4.[1] Radiograph of a twenty-day-old normal embryo for comparison with Fig. 5.

FIG. 5. Radiograph of a twenty-day-old embryo treated with 0.02 cc of benzyl alcohol on the fourth day of incubation, showing generalized developmental disturbance of bone resembling "pre-natal" type of osteogenesis imperfecta with defective ossification and associated deformities of the long bones of the limbs. These are obviously responsible for the "micromebia".

FIG. 6. Sagittal section of the tibia (×12) of a normal twelve-day-old embryo stained with toluidine blue. The cartilaginous core of tibia at the ends of the bone has been stained deeply lilac (by the metachromatic stain.) Ossification begins in the middle of the shaft and spreads towards the periphery, while marrow cavity forms by resorption of the cartilaginous core. For comparison with Fig. 7.

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[1] Acknowledgement is made to the following journals in which the figures designated have been published previously:

Brit. Med. J. 1950, **2:** 384–390 (Fig. 4)

J. Bone & Joint Surg. 1952, **34B,** No. 4: 646–698 (Figs. 6 and 8)

FIG. 4

FIG. 5

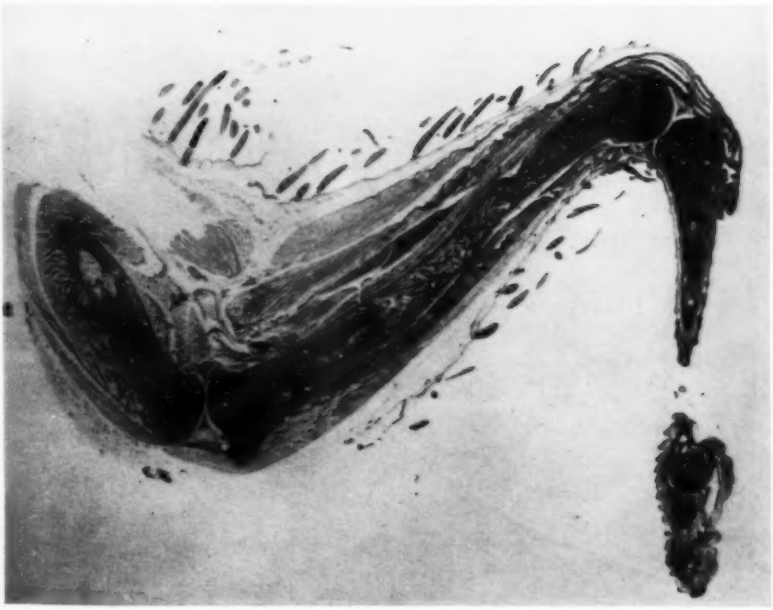

FIG. 6

FIG. 7. Sagittal section of the tibia (×25) of a twelve-day-old embryo treated with benzyl alcohol on the fourth day of incubation. Note the angulation deformity of the tibia at the site at which pseudarthosis of the tibia usually occurs in children. Compare with Figs. 6 and 8.

FIG. 8. Sagittal section of the tibia (×25) of a twelve-day-old embryo treated with insulin on the fourth day of incubation. Note the angulation deformity of the tibia and the severe degree of degeneration in its cartilaginous core. Compare with Fig. 7.

FIG. 7

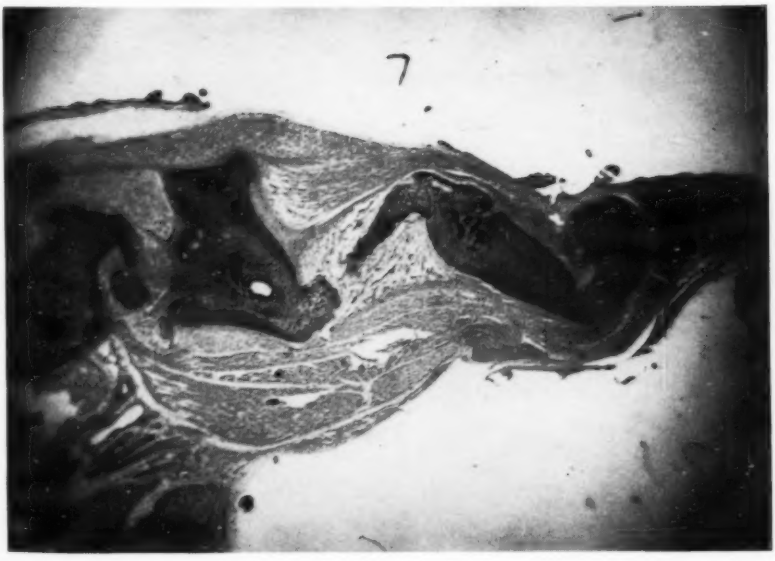

FIG. 8

FIG. 9. Sagittal section of the femur (×25) of a twelve-day-old embryo treated with benzyl alcohol on the fourth day of incubation. Note the bowing of the femur.

FIG. 10. Twenty-day-old embryos (middle and right) showing meningoceles and paralytic clawing of toes induced with 0.8 mgm. of lead nitrate injected into the yolk-sac on the fourth day of incubation. Compare with Fig. 1. Normal twenty-day-old embryo (left) for comparison.

FIG. 9

FIG. 10

A NEW TECHNIQUE FOR COMPARATIVE PHASE-CONTRAST AND ELECTRON MICROSCOPE STUDIES OF CELLS GROWN IN TISSUE CULTURE, WITH AN EVALUATION OF THE TECHNIQUE BY MEANS OF TIME-LAPSE CINEMICROGRAPHS[1]

EMIL BORYSKO[2] AND PETER SAPRANAUSKAS

Department of Parasitology, School of Hygiene and Public Health and Department of Surgery, School of Medicine, The Johns Hopkins University

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Comparative optical and electron microscope observations of the structure of whole mounts of cells grown in tissue culture (1, 2, 3) have suffered the disadvantage of being confined to the structure of the thin peripheral areas of the cells at the edges of the colonies. Even here, the mitochondria and other dense cytoplasmic constituents proved to be too thick for the resolution of their fine structure in the electron microscope. The limitations of the instrument require that thin sectioning be utilized to achieve this end. However, the delicate cells are firmly adherent to the glass substrate on which they are grown and harvesting them for sectioning presents a difficult technical problem. The technique presented here permits the embedding of whole colonies of cells in an optically clear matrix without removing them from the glass substrate. The cells can be examined with a phase-contrast microscope at any stage of the process and can subsequently be sectioned and examined in the electron microscope.

EMBEDDING TECHNIQUE

The cells selected for this work were from a strain of normal rat fibroblasts (designated as 14 p) which has been maintained in continuous culture for a number of years by Gey *et al.* (4). Cells, transplanted from roller tube cultures, were grown on No. 0 coverslips in stainless steel drilled slides (Diagram 1) in a medium consisting of 30 per cent cord serum in balanced salt solution. The drilled slides had been designed by Gey *et al.* for short term studies of cells (5). After incubating for about 24 hours, the colonies became firmly attached to the lower coverslip and were surrounded by numerous flat cells that had proliferated and migrated away from the original transplant. The upper coverslip was then removed and the depression of the drilled slide was filled with a 1 per cent solution of osmic acid buffered to pH 7.3 with a veronal buffer (6). After fixing for ten minutes, the osmic acid was carefully drained and the colonies, still attached to the coverslip, were washed, dehydrated and im-

[1] Supported by a Grant-in-Aid from the National Cancer Institute.

[2] Fellow of the Damon Runyon Memorial Fund for Cancer Research.

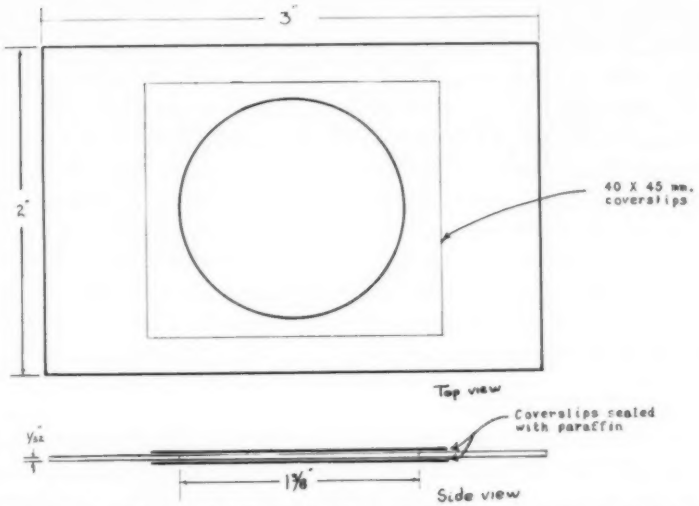

DIAGRAM 1. Stainless steel drilled slide. The cells were grown in the chamber formed by covering the hole in the center of the slide with No. 0 coverslips.

pregnated with methacrylate monomer in the following series of liquids: water, 70 per cent alcohol, 85 per cent alcohol, 95 per cent alcohol, absolute alcohol and finally, methacrylate monomer (7). The cells were kept in each liquid for ten minutes, remaining in the last change of monomer for ten minutes to two hours. The monomer used was a mixture of four parts of n-butyl methacrylate to one part of methyl methacrylate. To prevent evaporation, the upper coverslip was replaced after each change.

To embed the colonies, the cavity of the slide was filled with methacrylate that had been previously partially polymerized to a high viscosity, the upper coverslip was replaced and polymerization was carried to completion in an oven held at 45°C. The use of partially polymerized methacrylate at this stage of the process was found to be necessary to prevent the excessive evaporation that occurred during the final polymerization when stock monomer was used. Furthermore, it has been our experience that the use of partially polymerized methacrylate prevents damage to the tissue during polymerization and produces matrices with uniform hardness and solubility properties (8). The partial polymerization was carried out by filling a 150 x 15 mm. test tube with monomer to a depth of about 30 mm. and heating it in a water bath held at 70°C., agitating frequently to prevent layering. The methacrylate was considered to be sufficiently viscous when, upon holding the test tube at an angle of 45 degrees to the vertical with the bottom up, it took about ten seconds for the meniscus to reach the opposite end of the tube.

The final polymerization of the matrix was completed in about four hours though, for convenience, the preparations were allowed to remain in the oven overnight. The coverslips were then removed by gentle prying them away from the methacrylate with razor blades, taking care that the surfaces containing the colonies were not marred. Chilling the slides in ice water facilitated the removal of the coverslips. It should be emphasized that, for successful removal, the thinnest coverslips available should be used. When the coverslips were stripped off, all of the cells remained firmly embedded in the methacrylate. The embeddings were then trimmed and mounted for sectioning.

Sections for examination in the electron microscope were cut with glass knives (9) on a Servall microtome (10). An RCA EMU 2C microscope was used for all of the observations.

Results

The embeddings were examined in a phase contrast microscope both before and after removing the coverslips and it was found that this operation did not cause any damage to the cells. They were quite life-like in appearance and only the slight brownish tinge imparted by the fixative and the lack of Brownian motion served to distinguish them from living cells.

The sections examined in the electron microscope were all cut at right angles to the broad plane of the colonies, ensuring complete orientation with respect to the attached and free surfaces of the cells. Projections from the free surface of the cells, comparable to those seen on living cells, were readily identifiable (Fig. 1). These projections seemed to occur most frequently near the point of contact with neighboring cells. The appearance of the cells in the deep layers of the central portion of the colonies differed from that of the cells at the free surface. In the latter position, the cells were roughly cuboidal in cross-section but, with increasing depth, they became flattened in the horizontal plane (Fig. 3). In Figure 2, it can be seen that the technique has preserved the relationship of the cells to the glass substrate. Contact between the cell and the coverslip appears to be maintained by small projections from the cell rather than by a flattening of the entire plasma cell membrane against the glass.

EVALUATION OF THE TECHNIQUE

In order to determine whether artifacts were produced during the processing of the colonies for sectioning, four time-lapse phase-contrast cinemicrographs of selected cells were made through the entire procedure, from the living condition to the final embedded state. For this, a stainless steel perfusion slide (Diagram 2) was constructed in which the cells could be grown on coverslips in the usual manner and subsequently exposed to the processing fluids while under continuous observation in the microscope. The slide consisted of two plates joined together by small strips so that they were separated by a

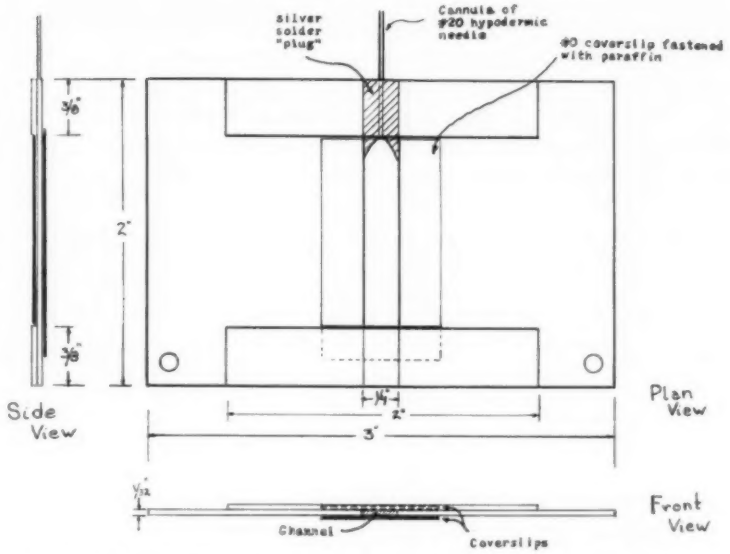

DIAGRAM 2. Stainless steel perfusion slide used for making time lapse cinemicrographs of the cells while they were being processed.

quarter-inch space. At one end of this space, the cannula of a No. 20 hypodermic needle was soldered in place with silver solder and a plug was formed level with the surfaces of the plates. Holes at the outer edges of the slide provided a means for fastening it rigidly to the stage of a microscope. Number 0 coverslips, cut to size, were cemented over the space between the plates with paraffin, forming a channel in which the cells could be grown and through which the various processing fluids could be passed. The open end of the channel and the cannula were sealed with paraffin while the cells were being grown. When thin cells appeared at the margins of the colonies, these paraffin seals were broken and a length of polyethylene tubing, fitted with the hub of a hypodermic needle, was attached to the cannula. The slide was then fastened to the stage of a phase contrast microscope with two small screws. The microscope was contained in a compartment in which the temperature could be regulated through the entire range required for the incubation of the cells and the polymerization of the embedding matrix (37° to 45°C.). A 1 cc. syringe, attached to the hub at the free end of the polyethylene tube, served to force the fixing, dehydrating and embedding fluids through the channel of the slide while time-lapse motion pictures (36 frames per minute) were made of the cells. Fluid ejected from the open end of the channel was absorbed with soft filter paper.

The temperature of the compartment was maintained at 37°C. while motion pictures of the living cells were being made. After fixation, the compartment was opened and kept at room temperature during the dehydration and impregnation of the cells. When the channel of the slide was filled with partially polymerized methacrylate, the compartment was closed and the temperature raised to 45°C. for the final polymerization of the matrix.

Results

Artifacts resolvable with the phase microscope could not be detected at any stage of the process. Figure 4 shows an area of a living cell as it appeared a few seconds before fixation. Figure 5 shows the same area of the cell in its final embedded state. The contours of the cell are well preserved and there is no evidence of shrinkage. The size, shape and position of the mitochondrial elements and other cytoplasmic structures have not been altered. The difference in contrast which is evident in the two pictures can partly be explained by the difference in the indices of refraction of the media surrounding the cells. However, it is possible that physical and chemical changes at the submicroscopic level also contribute to the altered optical properties of the preparation.

DISCUSSION

The technique presented here, based on the lack of adhesion between polymethacrylate resins and a polished glass surface, has a number of attributes that are highly desirable in the processing of tissue culture cells for study in the electron microscope. The danger of artifact production due to mechanical trauma has been almost completely eliminated. Surface contours of the cells are well preserved and artifacts which may be produced by the passage of a gas-liquid interface through the cells, as in the whole mount technique, are avoided since the colonies are not allowed to dry at any stage of the process. The optical clarity of the matrix permits the selection of specific groups of cells for study in the electron microscope. It is quite possible that the electron microscope and phase contrast images of specific structures within a particular cell may be compared by this method.

In the past, studies of the processing of cells for sectioning have been confined mainly to the effect of the fixative while the effects of dehydration, impregnation and embedding were almost completely ignored. It is now possible to study every phase of processing. By the use of time-lapse phase-contrast cinemicrography, a continuous record of the dynamic aspects of artifact production can be made and studied. It is hoped that this will lead to basic improvements in our present day techniques.

The technique need not be confined to the processing of cells grown on coverslips. Utilizing the principle of the lack of adhesion between polymethacrylates and glass surfaces, we have succeeded in embedding colonies of cells that had

been grown in roller tubes. This was done by simply fixing, dehydrating and impregnating the cells while they were attached to the walls of the roller tubes, after which the tubes were filled with partially polymerized methacrylate and the cells were embedded by completing the polymerization at 45°C. in the usual manner. When the matrix had hardened, the tubes were chilled in ice water, causing the methacrylate to separate from the glass by virtue of a slight difference in the coefficients of thermal expansion. The test tubes were then carefully broken away, leaving the cells embedded in the methacrylate. Colonies were cut out with a jewelers saw and trimmed and mounted in the usual manner for sectioning.

SUMMARY

1. A technique was developed for embedding whole colonies of tissue culture cells in methacrylate while they remained attached to the glass substrate on which they were grown. Electron micrographs of thin sections of the colonies illustrate the effectiveness of the technique.

2. A time-lapse, phase-contrast cinemicrographic study of the preparation of cells for sectioning, using osmic acid fixation, ethanol dehydration and methacrylate embedding, indicated that artifacts resolvable in the optical microscope were not produced at any stage of the process.

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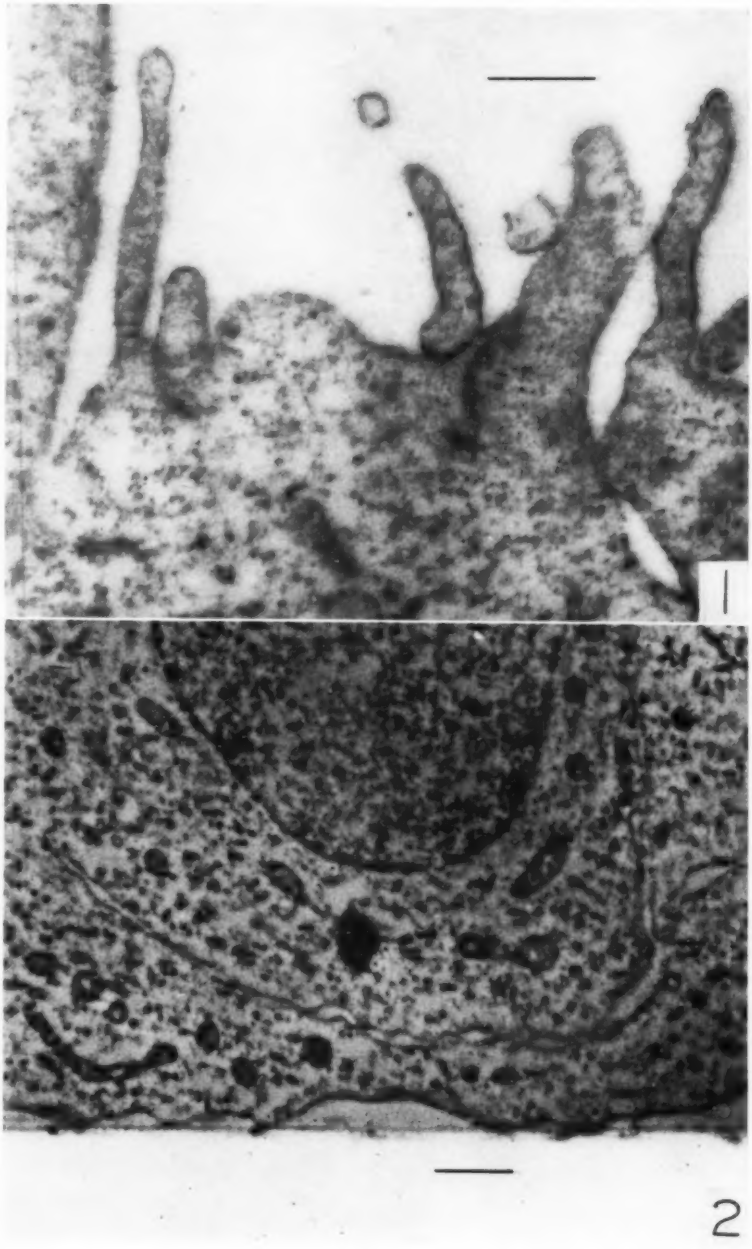
PLATE I.

Electron micrographs of sections of cells that had been embedded while they were attached to the glass substrate on which they were grown, illustrating the preservation of fine structure at the free and attached surfaces of the cells.

FIG. 1. Projections from the free surface of a cell resembling those which can be seen on the surface of living cells in the phase contrast microscope. Magnification 16,000×.

FIG. 2. Section through the basal portion of a cell. The methacrylate matrix (gray background material) was not removed from the section. It ends abruptly in a straight line where it had been lying against the coverslip. The surface of the cell does not appear to have been damaged by the stripping away of the coverslip and the manner of attachment of the cell to the coverslip by means of small projections is clearly revealed. Magnification 11,250×.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2

PLATE II

FIG. 3. Electron micrograph of a section of portions of two cells which were located about midway between the free and attached surfaces of the thick central region of a colony. The cells are flattened in a horizontal plane, parallel to the coverslip. They exhibit marked differences in mitochondrial size, the form of the "endoplasmic reticulum" and the size of the small cytoplasmic particles. Magnification 8,200×.

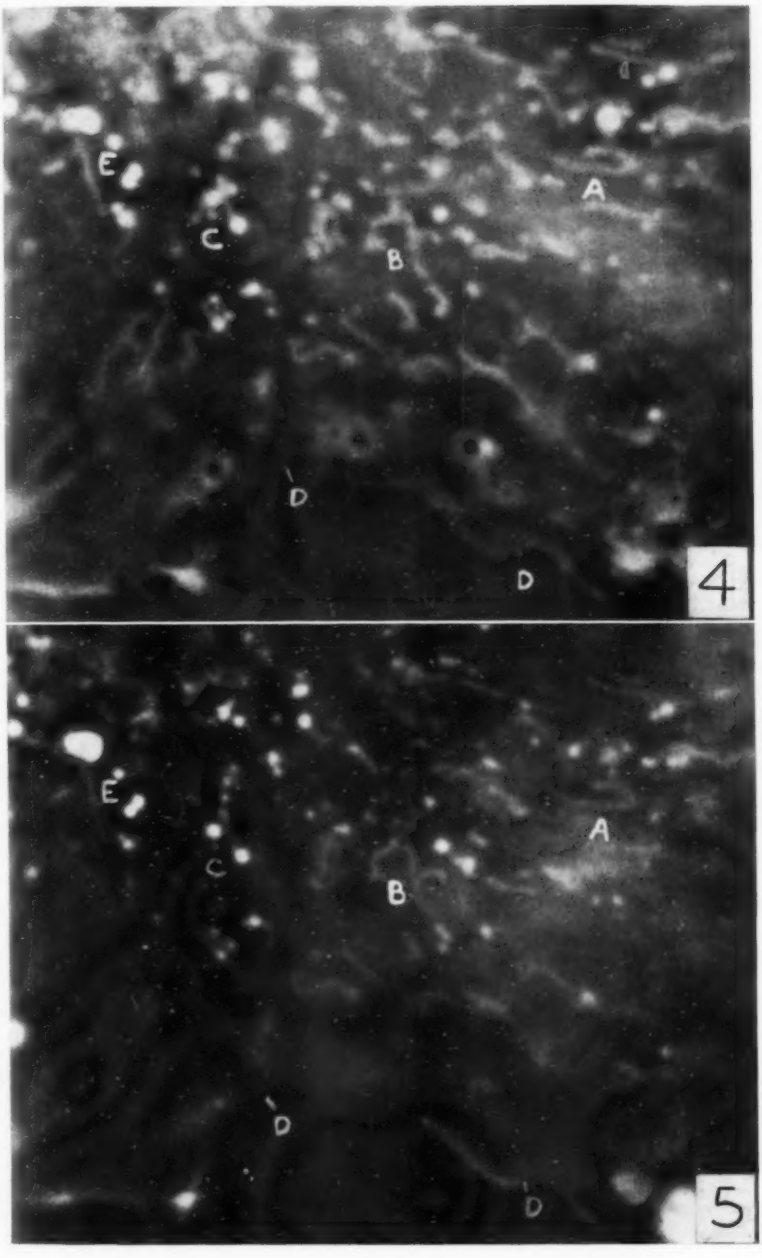
3

PLATE III.

Phase contrast photomicrographs taken from a time lapse movie of the processing of tissue culture cells for sectioning.

FIG. 4 shows a portion of a living cell as it appeared a few seconds before fixation with 1 per cent osmic acid.

FIG. 5 shows the same area as it appeared after fixation, dehydration and embedding in methacrylate. Mitochondria (A, B, C), fat droplets (C, E) and the cell boundary (D) have retained their form and relative positions throughout the entire process.


E
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5

THE EFFECTS OF PENTOBARBITAL ON THE METABOLISM OF ETHYL ALCOHOL IN DOGS

PHILIP WHITTLESEY[1]

Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine

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That the rate of disappearance of administered alcohol from the blood varies not only from individual to individual, but also from hour to hour and from day to day has been convincingly demonstrated by recent studies of Marshall and Fritz (1) in this laboratory in trained unanesthetized dogs. A question was raised as to whether or not closer control of the activity of the animal with a sedative agent would alter the rate of fall of serum concentrations of alcohol. Further, would such sedation alter the observed variability in the rate of decrease of serum alcohol in the individual animal from hour to hour and from day to day?

Since initial studies indicated, contrary to reports in the literature, a difference in the rate of decrease of serum alcohol concentrations between the untreated animal and the same animal anesthetized with pentobarbital, a more thorough investigation and analysis of these questions was carried out. The results are reported herewith. Data and analysis are presented to demonstrate that there is a decrease in the rate of disappearance of alcohol in the serum of dogs anesthetized with pentobarbital compared to that observed in the unanesthetized animal. This decrease is in the mean hourly rate of fall of serum alcohol; the variability from hour to hour cannot be shown in the present instance to be influenced by pentobarbital. Day to day variability in the overall rate of decrease of serum alcohol is less with pentobarbital anesthesia than without.

METHODS

Female mongrel dogs were used. Their diet consisted of Purina Dog Checkers and water *ad libitum*. For the experiments the dogs were neither thirsted nor fasted. The experiments were carried out over a period varying from two to three and a half months for the individual dog with an interval of at least forty hours elapsing between successive experiments. Room temperatures ranged from 22° to 26° C. When practicable, experiments with anesthesia were alternated with those in which no anesthesia was used. No randomization of experiments was used. In those experiments in which external heat was used it was supplied from two GE PS8A2 heating pads kept at 125° F. intermittently in order to maintain the rectal temperature at pre-anesthetic levels.

Forty-four experiments were performed on four dogs under the conditions outlined. A control specimen of blood was obtained at the outset. When anesthesia was used sodium

[1] Eli Lilly Fellow in Pharmacology and Experimental Therapeutics.

pentobarbital, 25 mg. per kg. in a 2.5 per cent aqueous solution, was injected intravenously. Supplements of 0.3–0.7 mg. per kg. were given when signs of restlessness appeared. In the doses used there were no clinically evident signs of depression of respiratory rate or amplitude. There was no apparent inhibition of spontaneous voiding or defecation. Subsequent to induction of anesthesia a 10 per cent solution of ethyl alcohol in normal saline was injected intravenously over a 5 to 8 minute period in a dose of 1.0 gm. per kg. One hour was allowed for distribution. Then a specimen of blood was drawn and again at 60 ± 1 minute intervals for four additional hours. These specimens were centrifuged immediately and refrigerated in 1 ml. glass-stoppered tubes. Alcohol determinations were run on the serum either at the conclusion of the experiment or the following day. Control studies showed no detectible loss of alcohol content during an overnight stand at 4° C. In those experiments in which no anesthesia was used the dog was kept in a small cage where its observed activities consisted of moving about (only occasionally), standing or lying quietly, or sleeping.

Alcohol determinations were done on three 0.02 ml. aliquots for each of the five hourly serum specimens by the method of Bücher and Redetzki (2). The optical density of the reduced diphosphopyridine nucleotide was measured in a Beckman DU spectrophotometer at 340 mμ. Slit widths varied from 0.18 to 0.22 mm. at maximum sensitivity. Optical density readings ranged from 0.150 to 0.850. The samples were read, as seen in the accompanying table, in blocks of three (designated by letter) from left to right in rows from top to bottom against a distilled water blank. The cuvette order (designated by subscript numeral) was unchanged throughout. Drift errors of the instrument as well as cuvette errors were balanced out by this method of reading. These variations were of small magnitude and are neglected in the estimation of reading errors. The serum alcohol estimations were made by applying each aliquot reading to a rectilinear calibration curve determined each day from standards covering the expected range of values. These calibration curves did not vary greatly, showing a maximum displacement range of 8 mg. per cent and a range of slopes of 21.7 to 22.7 mg. per cent per 0.1 O.D. unit. A specimen of serum obtained one hour after injection of the usual dose of sodium pentobarbital showed no interference in the determination of alcohol when compared with standard alcohol solutions.

ALIQUOT	HOUR SPECIMEN				
	1	2	3	4	5
1	A_1	A_2	A_3	B_1	B_2
2	B_3	C_1	C_2	C_3	D_1
3	D_2	D_3	E_1	E_2	E_3

RESULTS

The data obtained from forty-four experiments on four dogs with and without the use of pentobarbital anesthesia have been summarized in Table I. The chief analytical tool in the evaluation of these data has been a standard analysis of variance (3). The best fitting straight-line regression slope of the sera alcohols, $\hat{b}$, *i.e.* the hourly decrease in alcohol concentration, for each experiment has been estimated by the method of least squares (3). The range of values of $\hat{b}$ obtained in the unanesthetized dog agrees with that observed by other investigators.

Table IIA indicates the mean hourly rate of decrease of alcohol concentration, b, for each dog with and without pentobarbital anesthesia. The mean $\hat{b}$ for dogs with pentobarbital averages 2 mg. per cent per hour less than the mean $\hat{b}$ for the same dogs unanesthetized. This is a decrease of about 10 per cent in these experiments. Ninety-five per cent confidence limits set in the usual manner show that this average difference lies between 0.7 and 3.4 mg. per cent per hour. The slopes, $\hat{b}$, were analyzed for their variability from dog to dog with and without pentobarbital as well as for the interaction effect between dog and anesthesia as shown in Table IIB. The residual measures the day to day variation in the slopes for the same dog under otherwise constant conditions. Despite the fact that slopes derived from data on various dogs show substantial differences between dogs, it is clear that there is a consistent reduction in slopes with pentobarbital. The lack of interaction indicates that

TABLE I

Data on Serum Alcohol Concentrations Following the Administration of Ethyl Alcohol WITHOUT PENTOBARBITAL

DOG WT.	EXPT.	HOURLY AVERAGE SERUM ALCOHOL CONCENTRATIONS IN MG.%					$\hat{b}$†	$\hat{e}$	$s^2_{\hat{b}/a}$	$s^2_{y.x}$	$s^2_{y.x^2}$	$s^2_{\hat{b}}$
		1	2	3	4	5						
							mg%/hr	*mg%/hr²*	*(mg%)²*	*(mg%)²*	*(mg%)²*	*(mg%/hr)²*
Velva	1*	107.20	90.17	69.80	53.70	37.27	17.63	0.391	0.588	1.724	1.518	
kg.	3*	83.23	65.47	48.60	31.23	15.17	17.04	0.207	0.264	0.255	0.082	
13.4 to 15.0	5*	85.00	62.47	45.13	28.47	12.67	17.87	1.010	0.270	6.681	2.881	
	7*	88.30	67.33	47.90	28.00	11.87	19.22	0.658	0.521	2.371	0.528	
	9*	91.20	74.97	61.07	45.30	31.37	14.93	0.195	1.550	0.483	0.459	
Means							17.34	0.492	0.639	2.303	1.094	2.536
Kabish	2	147.97	127.60	107.63	90.57	69.53	19.39	0.112	0.901	1.119	1.590	
kg.	4	128.30	113.03	98.23	81.10	63.67	16.39	−0.496	0.660	1.250	0.150	
16.6 to 18.2	6	132.80	116.33	102.40	93.13[a]	74.50[b]	15.07	0.550	0.802	0.336	1.489	
	8	120.57	105.30	85.27	66.93	49.57	18.04	−0.178	0.493	1.490	2.014	
	10	124.27	105.13	88.13	68.26	53.33	17.88	0.396	1.265	1.777	1.566	
Means							17.35	0.077	0.824	1.194	1.362	2.760
Ara	1*	79.77	57.57	38.57	21.27	7.13	18.16	1.272	0.252	7.603	0.063	
kg.	3	150.90	133.77	114.37	97.10	76.07	19.02	−0.424	0.054	1.416	0.868	
8.7 to 11.4	8	112.53	90.07	66.73	43.83	23.13	22.50	0.283	1.017	0.691	0.476	
	9	152.87	126.93	111.57[c]	85.17[d]	61.63[e]	24.27	−0.314	1.212	1.635	2.666	
	10	140.07	118.13	103.07	83.00	66.93	18.14	0.481	1.196	3.435	3.535	
Means							20.42	0.260	0.746	2.956	1.522	7.854
Dirne	1*	101.87	75.73	52.60	31.20	16.60	21.51	1.772	0.248	15.327	1.006	
kg.	3	120.47	87.97	64.10	39.10	17.47	25.49	1.472	0.311	12.004	2.836	
13.5 to 16.0	5	121.67	100.70	79.97	63.23	47.40	18.60	1.019	0.516	5.086	0.356	
	8	153.13	131.17	110.87	90.63	72.40	20.20	0.537	0.535	1.410	0.097	
	10	138.33	117.40	95.73	76.87	56.93	20.33	0.342	0.688	0.814	0.406	
Means							21.22	1.028	0.460	6.928	0.940	6.653

[a] 3.6 hours. [b] 4.84 hours. [c] 2.75 hours. [d] 3.75 hours. [e] 4.75 hours.

Data on Serum Alcohol Concentrations Following the Administration of Ethyl Alcohol

WITH PENTOBARBITAL

DOG WT.	EXPT.	HOURLY AVERAGE SERUM ALCOHOL CONCENTRATIONS IN MG.% 1	2	3	4	5	b	$\bar{c}$	$s^2_{b/s}$	$s^2_{y.x}$	$s^2_{\bar{y}.x^2}$	s^2_b
							$mg\%/hr$	$mg\%/hr^2$	$(mg\%)^2$	$(mg\%)^2$	$(mg\%)^2$	$(mg\%/hr)^2$
Velva	2*	92.23	79.10	63.03	45.53	33.37	15.13	0.036	0.231	2.423	3.626	
kg.	4*	84.60	75.26	57.97	43.17	26.80	14.77	−0.826	1.063	5.212	3.537	
13.4 to 15.2	6*	91.27	76.20	62.70	49.40	32.07	14.52	−0.309	0.254	1.529	1.626	
	8*	88.47	73.03	53.33	40.73	23.00	16.32	0.180	0.447	2.826	4.013	
	10*	98.33	84.47	68.97	54.43	39.27	14.82	−0.119	0.343	0.182	0.125	
Means							15.11	−0.208	0.468	2.434	2.585	0.503
Kabish	1	146.07	124.70	110.37	94.93	79.37	15.71	0.293	1.182	1.134	1.199	
kg.	3	144.30	129.40	113.07	96.27	83.27	15.52	0.238	0.235	1.228	1.446	
15.0 to 17.9	5	130.27	115.33	103.80	85.37	67.77	15.50	−0.873	0.164	5.327	2.657	
	7	135.13	123.03	109.17	93.53	76.23	14.73	−0.870	1.490	3.533	0.027	
	9	127.80	107.23	90.20	72.13	54.57	18.16	0.356	0.721	1.057	0.727	
Means							15.92	−0.171	0.758	2.456	1.211	1.704
Ara	2*	84.96	68.57	52.10	35.57	20.37	16.23	0.159	0.279	0.208	0.137	
kg.	4	153.93	133.30	110.40	85.33	65.10	22.56	−0.098	1.340	2.409	3.547	
8.9 to 10.5	5	138.63	120.46	101.43	87.17	68.90	17.28	0.326	1.146	1.817	1.979	
	6	140.13	125.10	103.57	82.97	64.03	19.43	−0.492	3.305	3.564	3.650	
	7	124.77	107.17	85.13	66.27	46.57	19.75	−0.073	0.391	1.102	1.616	
Means							19.05	−0.036	1.292	1.820	2.186	6.003
Dirne	2	145.73	130.37	107.73	87.37	69.30	19.59	−0.224	0.495	3.722	5.231	
kg.	4	137.27	123.73	102.40	83.63	64.67	18.53	−0.591	1.988	4.375	4.114	
12.7 to 13.4	6	142.27	124.23	106.03	91.83	74.13	16.87	0.334	0.752	1.541	1.529	
	7	156.47	139.70	124.93	108.47	90.27	16.36	−0.354	0.501	1.022	0.658	
	9	146.10	132.10	108.70	91.53	72.46	18.78	−0.278	1.773	4.926	6.849	
Means							18.02	−0.223	1.102	3.117	3.676	1.845

WITH PENTOBARBITAL PLUS HEAT

Velva	15	153.63	138.17	123.33	108.30	90.83	15.55	−0.301	1.248	0.744	0.483	
kg.	19	146.73	129.17	115.53	100.87	82.10	15.76	−0.246	0.878	2.447	3.249	
14.8 to 15.5												
Means							15.66	−0.274	1.063	1.596	1.866	
Dirne	12	150.63	128.17	110.23	90.23	71.57	19.61	0.346	0.493	1.542	1.237	
kg.	16	150.37	131.33	112.53	91.73	73.43	19.35	−0.037	0.924	0.373	0.550	
13.2 to 14.2												
Means							19.48	0.155	0.708	0.958	0.893	

* 0.6 gm./kg. alcohol and 30 mg /kg. sodium pentobarbital

† Headings of Table I

b = rate of decrease of serum alcohol, *i.e.* slope. It is estimated from the equation

$$b = \frac{-\sum_{i=1}^{5}(x_i - \bar{x})(y_i - \bar{y})}{\sum_{i=1}^{5}(x_i - \bar{x})^2}$$

TABLE II

A. Average Rate of Decrease in Serum Alcohol Concentrations, $\bar{b}$, for Each Dog in Milligram Per Cent Per Hour

	VELVA	KABISH	ARA	DIRNE
Pentobarbital	15.11	15.92	19.05	18.02
No pentobarbital	17.34	17.35	20.42	21.22

B. Analysis of Variance of $\bar{b}$, Average Rate of Decrease in Serum Alcohol Concentrations

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F
Pentobarbital	42.29	1	42.29	11.46**
Dogs	106.38	3	35.46	9.50**
Interaction	5.49	3	1.83	0.49
Residual	119.43	32	3.73	

** Significant at 1% level.

the reduction in slope due to pentobarbital is the same for all dogs within the limits of sampling error.

Having established that pentobarbital effected a decrease in the rate of fall of serum alcohol concentration, an inquiry was conducted into factors and errors possibly accounting for the observed difference. It was observed that during pentobarbital anesthesia there was a drop in rectal temperature of 2 to 3°C. To investigate the possible rôle of this decrease in body temperature on the observed rates of decrease of serum alcohol, two experiments were done

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where x_i denotes hourly intervals, y_i the mean hourly serum alcohol concentrations in mg.%/hr.

$\hat{c}$ = rate of change of rate of decrease of serum alcohol concentrations in mg.%/hr.². It is estimated for standard least square methods for fitting an equation of the form $Y = a+bx+cx^2$ (see Fisher) (3).

$s^2_{\bar{y}_i}$ = error of chemical determinations. It is estimated from the formula

$$s^2_0 = \frac{\sum_{i=1}^{5}\sum_{j=1}^{3}(z_{ij} - y_i)^2}{5 \times 2}$$

where z_{ij} denotes individual aliquot serum alcohol values in mg.%, y_i the average value of the three aliquots of a single serum specimen, 5 the number of specimens, and 2 the degrees of freedom in each group of aliquots.

$s^2_{y \cdot x}$ = variations about the regression line, $\bar{b}$. It is estimated from the formula

$$s^2_{y \cdot x} = \frac{\sum[(y_i - \bar{y}) + \bar{b}(x_i - \bar{x})]^2}{n - 2}$$

where $\bar{b}$ is the estimated slope, x_i the hour interval, y_i the aliquot alcohol value, and $n - 2$ the degrees of freedom.

$s^2_{y \cdot x^2}$ = variation about the quadratic regression curve. It equals the sum of squares of deviations from the fitted parabola divided by $5 - 3$ since three constants were estimated.

$s^2_{\bar{b}}$ = variance of regression slopes, $\bar{b}$ It is estimated from the equation

$$s^2_{\bar{b}} = \frac{\sum \bar{b}^2 - \frac{(\sum \bar{b})^2}{5}}{4}$$

where $\bar{b}$ is the regression slope, *i.e.* the rate of decrease of serum alcohol per hour.

on each of two dogs using pentobarbital with the addition of external heat to maintain the rectal temperature at pre-anesthetic levels. The results of these experiments are summarized in Table I. It may be seen that the slopes, while characteristic for each dog, do not increase beyond the range observed under pentobarbital alone and do not return to levels observed without anesthesia. The magnitude of errors is consistent with that obtained in the other studies. It does not appear, therefore, that the drop in body temperature can account for the decrease in rate of fall of serum alcohol.

The errors of measurement include, in addition to the individual error of each determination, an error common to each set of three readings. This component of error is thought to be small and no adjustment has been made for the resulting slight dependence between the estimates. The variability of the pooled errors of the chemical determinations appear in the columns $s^2_{0/3}$ in Table I. s^2_0 is a measure of the variation of the three determinations; each made on the same specimen of serum. The variance of the average of three such determinations is, therefore, $s_{0/3}$ (3) and is estimated for each experiment. The variability of the mean hourly alcohol concentrations about the fitted regression line is measured by $s^2_{y\cdot x}$, and is also determined for each experiment as shown in Table I. If the deviations of the points from a straight line were due solely to analytical errors of measurement then $s^2_{y\cdot x}$ should be equal to $s^2_{0/3}$ apart from random fluctuations. It may be observed in Table I that $s^2_{y\cdot x}$ tends quite consistently to be greater than $s^2_{0/3}$. Therefore, the deviations from linearity are greater than can be ascribed solely to the direct analytical errors of measurement. Additional deviations, however, are of the same order of magnitude as analytical measurement errors and are, at least in part, ascribable to small timing errors and other errors incident to the collection of the serum specimens. An analysis of variance of $s^2_{y\cdot x}$ gave no indication that the variation about the regression slope changed appreciably from dog to dog nor did it appear to be influenced by the presence of pentobarbital.

The variability of slopes from day to day, s^2_b, for each dog with and without pentobarbital is shown in Table I. If variation about the regression line could alone account for the variability of the $\hat{b}$'s then s^2_b should be of the same order of magnitude of $s^2_{y\cdot x/10}$ or approximately 0.2–0.3. Clearly the day to day variability of the slopes, s^2_b, is too great to be ascribed to this source alone. It is to be noted that for each dog the day to day variability of the rate of decrease of serum alcohol, s^2_b, varies less with pentobarbital than without.

To investigate the question of possible decrease of rate of disappearance of alcohol from the serum with decreasing levels of alcohol a quadratic regression was fitted to the data for each experiment. The quadratic coefficient, $\hat{c}$, for each experiment is shown in Table I. If curvature were present the mean square deviations from the fitted parabola, $s^2_{y\cdot x^2}$, should tend to be less than

$s^2_{y \cdot z}$ (3). For the experiments done with pentobarbital there was essentially no decrease whatever in the mean squares, indicating that there was no apparent tendency toward curvature. In the unanesthetized dog, however, a substantial reduction in mean square occurred which is reflected by the clear evidence of curvature in certain of the experiments, notably Dirne 1, 3, 5; Velva 2, and Ara 1. The mean quadratic coefficients, $\hat{c}$, for each dog with and without pentobarbital anesthesia are shown in Table 1. Thus, although there is no evidence of curvature in the dogs with pentobarbital, there does appear to be curvature, particularly in the instances noted above, in the unanesthetized dogs. It is of interest that these experiments were, almost without exception, those for each dog in which the lowest final levels of serum alcohol occurred.

DISCUSSION

One possible major source of error, in addition to the effect of temperature which has been examined experimentally, that might account for the observed difference in the rate of decrease of serum alcohol in the dog treated with pentobarbital compared to the rate in the unanesthetized state would be the difference in excretion of alcohol via the lungs and kidneys in the two states. These were not determined in this series of experiments. However, using calculations based on the findings of Marshall (4), Harger (5), Liljestrand and Linde (6), and Widmark (7), it may be estimated that a resting fifteen kilogram dog having a 150 mg. per cent alcohol blood level might exhibit a loss of as much as 0.3–0.4 mg. per cent per hour in the serum via the urine and expired air. Hence, even if the dog under pentobarbital were to cease breathing and cease excreting urine, the observed decrease in the rate of fall of alcohol in the serum during pentobarbital anesthesia could not be accounted for in major degree by the predicted savings.

It is of interest that Brody and Bain (8) have demonstrated *in vitro* an uncoupling of oxidative phosphorylation by pentobarbital in molar concentrations equivalent to those used in this study of 5 to 9 per cent. The extent to which pentobarbital may alter the metabolism of alcohol *in vivo* through this mechanism is not known.

Eggleton (9) concluded that "comparison of results obtained on animals (dogs and cats) under light nembutal (pentobarbital) anesthesia (.65 cc. per kg., equivalent to 40 mg. per kg., intraperitoneally) with those of other workers on unanesthetized animals shows that this anesthesia has little, if any, inhibitory action on the metabolic rate of alcohol." Loomis (10) drew similar conclusions from eight experiments on four dogs though no data were presented. The magnitude of the decrease in rates of fall of serum alcohol with pentobarbital compared to the unanesthetized state and the variation of these

rates from dog to dog appears to be such that Eggleton and Loomis would have been unable to make a clear distinction between them without larger series of controlled studies than they reported.

The variability of the hourly alcohol serum concentrations about the fitted regression slope in the present study confirms the findings of Marshall and Fritz (1), Bernhard and Goldberg (11), and Schønheyder *et al.* (12) in this regard. It is of interest that the regression slopes in the present study in the unanesthetized dogs were steeper than those observed by Marshall and Fritz (1) in their fasted dogs.

SUMMARY

Controlled studies on the rate of decrease of administered alcohol in the sera of a group of dogs have been made with and without pentobarbital anesthesia. An analysis of the data obtained discloses that:

1. Pentobarbital anesthesia in the doses used effects a reduction averaging between 4 per cent and 18 per cent in the rate of decrease of serum alcohol. This reduction cannot be ascribed to the decrease in body temperature or to a decrease in excretion of alcohol via the lungs and kidneys during pentobarbital anesthesia.
2. Pentobarbital reduces the day to day variability in the rate of decrease of serum alcohol.
3. The hour to hour variability in the rate of decrease of serum alcohol is essentially unaltered by pentobarbital.
4. The rate of decrease of serum alcohol in unanesthetized dogs shows a slight but significant tendency to decrease with decreasing serum alcohol concentrations. However, no significant departure from linearity could be ascertained under the influence of pentobarbital at the doses used over the range of alcohol values obtained.

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SPECTRAL PHONOCARDIOGRAPHY: CLINICAL STUDIES

VICTOR A. McKUSICK, GEORGE N. WEBB, JAMES R. BRAYSHAW AND SAMUEL A. TALBOT

Department of Medicine, The Johns Hopkins Hospital and Johns Hopkins University School of Medicine

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INTRODUCTION

The principles of sound spectrography and the instrumental modifications necessary for application of the method to phonocardiography have been presented previously (1). Figures 1 and 2 review these principles. In essence, sound spectrography involves the passage of sound (in electrical form) through a bank of filters each of which is tuned to a specific pass-band. The output from each filter activates the marking unit of the recorder which in Figure 1 is represented as a tiny light. Note that *time is on the abscissa* and *frequency spectrum on the ordinate* (here linear). *Intensity* (*loudness*) *is indicated by the brightness* (or blackness) of any given part of the record, inasmuch as the brightness of each bulb is a function of the filter output, which in turn represents the energy in a particular frequency band.

In constructing an instrument of the type just described, it is obviously expensive and difficult to provide the large number of individual filters necessary for high frequency resolution. Therefore, the sound spectrograph employed in these studies has been developed along the lines indicated in Figure 2. Recordings are first made on magnetic tape. These are then edited and desirable sections picked out for analysis. Such a section is played onto a loop on the rim of the disc which is mounted on the same axis as the kymograph drum. This segment of sound is then played back repeatedly through an electronic device which *in effect* is a single filter the tuning of which is changed progressively with the repeated playbacks. Recordings are made on electro-sensitive paper. A mechanical link provides for progressive change in the *effective* tuning of the filter set-up as the writing arm moves up the screw. It should be appreciated that what is in essence the single filter has not only a variable center pass-band frequency but also an adjustable pass-band width. The important considerations of selection of pass-band width in spectral phonocardiography are discussed in the previous communication (1).

METHODS

For pick-up from the patient, a condenser-type microphone (Altec 21BR150) has been employed. This is a calibrated transducer with linear characteristics. Recordings were made

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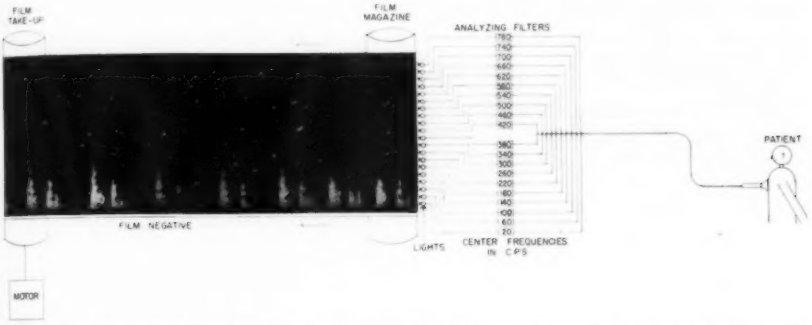
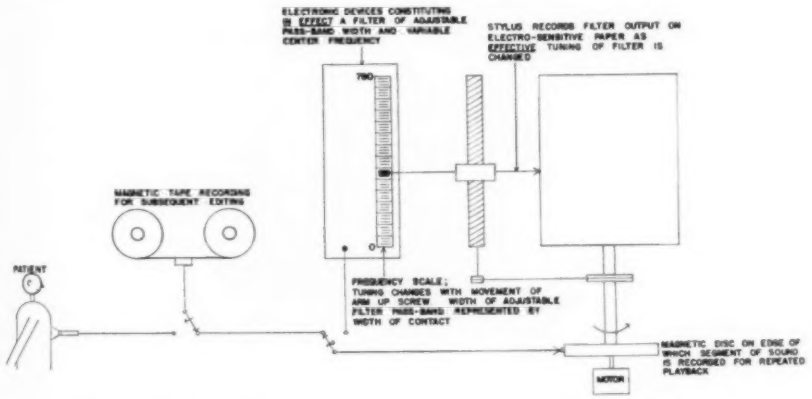


FIG. 1. The principle of a sound spectrograph which provides direct (i.e. instantaneous) analyses.


ELECTRONIC DEVICES CONSTITUTING IN EFFECT A FILTER OF ADJUSTABLE PASS-BAND WIDTH AND VARIABLE CENTER FREQUENCY
STYLUS RECORDS FILTER OUTPUT ON ELECTRO-SENSITIVE PAPER AS EFFECTIVE TUNING OF FILTER IS CHANGED
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MAGNETIC TAPE RECORDING FOR SUBSEQUENT EDITING
PATIENT
FREQUENCY SCALE; TUNING CHANGES WITH MOVEMENT OF ARM UP SCREW WIDTH OF ADJUSTABLE FILTER PASS-BAND REPRESENTED BY WIDTH OF CONTACT
MAGNETIC DISC ON EDGE OF WHICH SEGMENT OF SOUND IS RECORDED FOR REPEATED PLAYBACK
MOTOR

FIG. 2. The principle of the sound spectrograph used in these studies.

under as quiet ambient conditions as possible. The microphone-chest piece combination employed provides a maximum of 20 db attenuation of ambient noise. The patient is essentially isolated from floor vibrations by an air mattress.

An Ampex two-channel, type 350 recorder has been employed. Recordings have been made at 7½ inches per second. To record the full dynamic range of the heart sounds, three degrees of low frequency compression have been available. With no compression the response characteristics of the entire system are flat above 30 cycles. At 20 cycles the response has dropped 5–10 db. and at 15 cycles has dropped a maximum of 20 db. below the response level above 30 cycles.

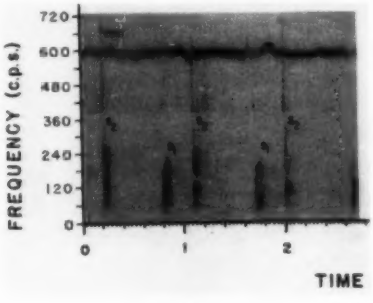
In the analyzing step, the filter pass-band width which was found to give the best compromise definition in both time and frequency was approximately 23 c.p.s. at 6 db. attenuation below the intensity of the center frequency (1). (Occasionally when the information desired was of a special variety other widths of pass-band might be employed, e.g. a wider one for particularly sharp time definition.) A 750 c.p.s. frequency range was adequate in the majority of cases although a 1500 c.p.s., or higher, scale was occasionally necessary to encompass the total frequency range encountered. *In essence*, then, we have analyzed these

sounds through approximately 425 overlapping filters, each with its center frequency about 1.8 c.p.s. from the next and each with a band width of approximately 23 c.p.s. in terms of the definition given above.

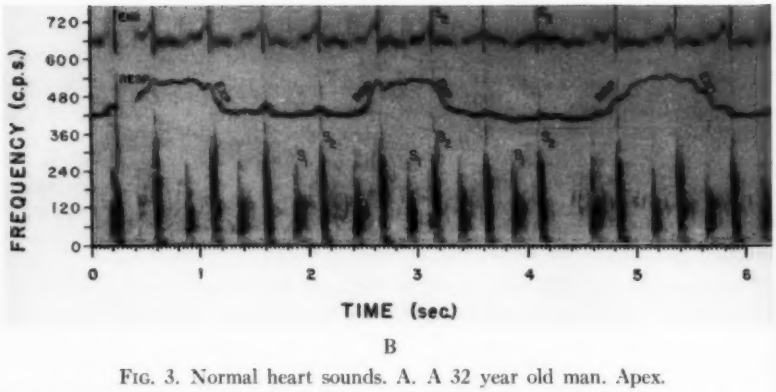
The frequency scale (ordinate) can be made logarithmic with advantageous spreading of the lower frequency range where maximum information is concentrated. (For an example of a logarithmic frequency display see Figure 10B). In the analysis of the normal heart sounds in particular this type of display is useful since their content tends to be limited largely to the range below 150 cycles. The logarithmic frequency scale has been accomplished by alterations in the characteristics of the mechanical link that changes the effective tuning of the analyzing filter as the writing arm moves up the drum. It may be that the logarithmic display will prove to be more realistic, that is, will resemble more closely the mental image of the sounds, and will in general give more information than a more expanded linear display. Our experience with both the logarithmic display and expanded linear displays (with a frequency range to 300 cycles, for example) is thus far limited.

The presently used spectrograph produces a record with a four-inch frequency scale (ordinate). A segment of sounds 6.6 seconds in duration is displayed on a record 12½ inches long.

We are not concerned here with precisely quantitative intensity considerations. Suffice



A



B

FIG. 3. Normal heart sounds. A. A 32 year old man. Apex.

B. A 5 year old boy. Pulmonary area. Note the splitting of S2 during part of the respiratory cycle.

it to say that since the microphone is calibrated the introduction of a calibrating signal, or signals, just beyond the microphone permits quantification of the intensity dimension and expression of intensity in any given frequency band in terms of dynes per sq. cm. of sound pressure.

For timing purposes the EKG and the respiration mark has been put directly on the spectral phonocardiogram by means of frequency modulated carriers. (See Figures 3A and B and 14A for examples).

In the original publication (1) five filter systems were characterized as to the resolution in time and in frequency afforded by each. The relative time lag of each of these filter systems has been investigated. The filter system with narrowest pass-band (A) produces a record which not only persists longer but also starts later than the record produced by the filter system of widest pass-band (E). The time lag of filter system A as compared with E is of the order of .015 seconds.

THE NORMAL HEART SOUNDS

A subsequent communication will present detailed descriptive and quantitative spectral phonocardiographic studies in a group of normal young adult subjects. As demonstrated by the two records in Figure 3, the majority of the energy content of the heart sounds lies below 200 c.p.s. Children with thin chests may show appreciable representation above this level (20). How much of this is the result of particular vigor of the heart beat, i.e., the "snappiness" of the generator, and how much due to the characteristics of the chest walls is difficult to know. Intrathoracic recordings may be helpful in this connection. As will be discussed later, intensity and range of frequency spectrum tend to parallel each other closely. The louder the sound, the higher is the peak frequency, as a rule. Slight splitting of heart sounds is frequently encountered as a normal variation.

SOME ALTERATIONS IN THE HEART SOUNDS THEMSELVES

The appearance or exaggeration of splitting of P2 at the end of inspiration and beginning of expiration and the usefulness of this phenomenon in studying

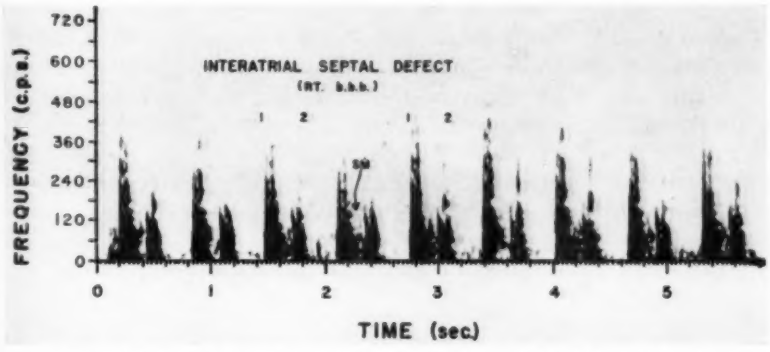


FIG. 4. Split heart sounds with right bundle branch block (aortic area).

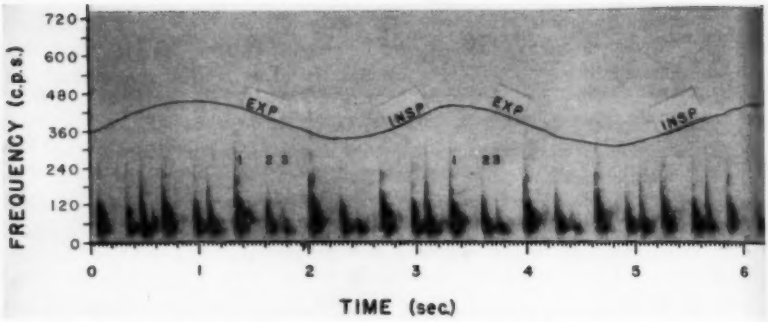


FIG. 5. Normal third heart sound with respiratory variations. Sinus arrhythmia.

the genesis of the constituent parts of the second heart sound, has been re-emphasized by Leatham (16). (See Figure 3B for an example of this phenomenon.)

Figure 4 demonstrates the splitting of both heart sounds which occurred in a 34 year old patient (M. S., 324542) with inter-atrial septal defect producing pulmonary hypertension and right bundle branch block. The second component of the split—that contributed by the right side of the heart—is louder and in most cycles has higher frequency components. (See Figure 15 for a similar case.) Similar splitting of the heart sounds accompanying ventricular extrasystoles is well demonstrated by this method. Luisada (23) states that a split of 0.06 seconds is about the minimal interval detectable by the human ear. The split in Figure 4 is of this order of magnitude and was barely perceptible by ear.

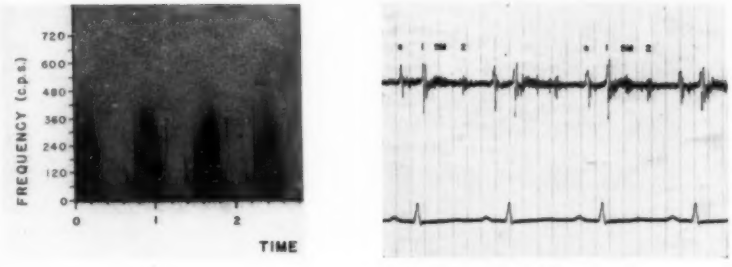
NORMAL THIRD SOUND

Figure 5 demonstrates an unusually conspicuous third heart sound in a young subject. There is sinus arrhythmia and rather striking respiratory variation in the third heart sound. At the height of expiration the first heart sound is loudest and the third sound faintest. The accentuation of the third sound during inspiration is of physiological interest. This accentuation may be the result of an increase in ventricular filling, specifically right ventricular filling. Compare the accentuation which occurs with exercise and the diminution with the application of tourniquets to the limbs (18).

GALLOPS

In Figure 6A is presented a spectral phonocardiogram from a patient (J. T., 276556) who appears to have an interventricular septal defect. There is also an atrial gallop. In Figure 6B the same sounds are displayed as a conventional phonocardiogram.

In Figure 7 is presented the recording of a 15 year old boy (F. C., 585527)



A B

FIG. 6. Interventricular septal defect with atrial (presystolic) gallop. A. Spectral phonocardiogram. B. Conventional phonocardiogram.

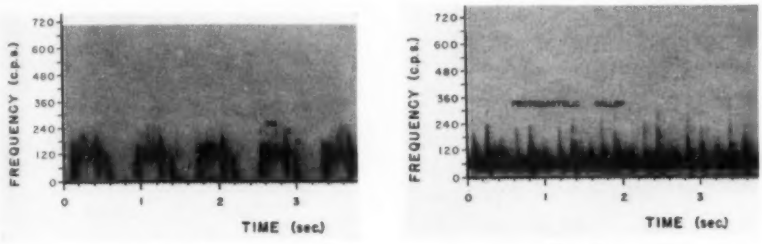


FIG. 7 FIG. 8

FIG. 7. Rheumatic mitral regurgitation with protodiastolic sound.
FIG. 8. Protodiastolic gallop. A systolic murmur is present.

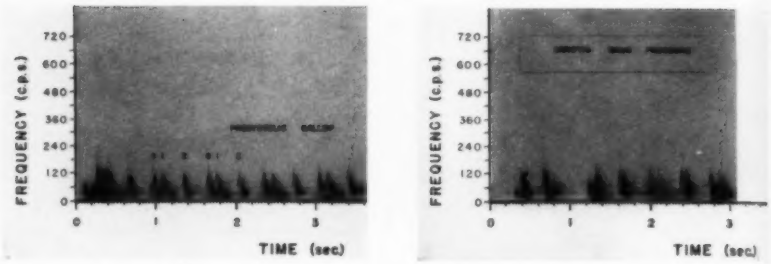
with rheumatic mitral valvulitis producing mitral regurgitation. A very large proportion of patients with mitral regurgitation as the sole or predominant lesion develop a prominent third sound, or protodiastolic gallop (24). Such is the case in this patient.

Figure 8A and B presents a gallop which, when the heart is slowed by carotid sinus pressure, is seen to be presystolic in timing. Levine and Harvey (5) have pointed out that a convenient method for timing gallops is to slow the heart by carotid sinus pressure and see whether the gallop sounds "stays with" the first sound (a presystolic gallop) or the second sound (a protodiastolic gallop). Also demonstrated are the characteristics of heart sounds which are said to be of poor quality. Note the low frequency content. It appears that the heart does not have the snapping action responsible for the presence of components of higher frequency in normal heart sounds.

Figure 8 presents an instance of protodiastolic gallop.

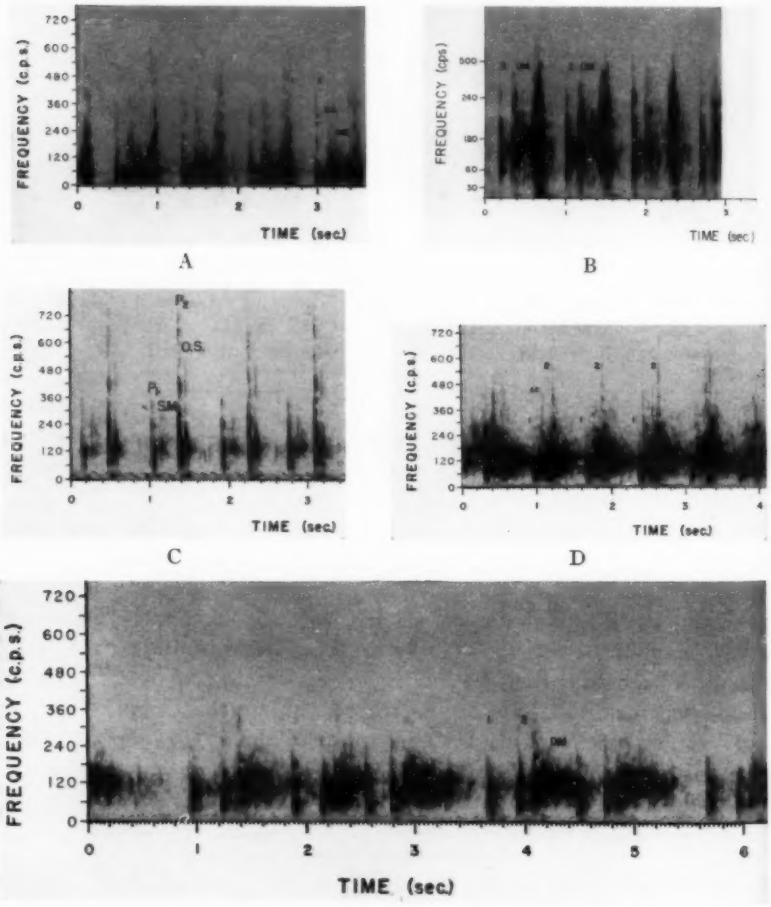
MITRAL STENOSIS

Twenty cases of mitral stenosis were surveyed. Representative cases are presented in Figure 10. Notice in A that the ringing character of the first sound



A B

FIG. 9. A. Presystolic gallop. B. Same with carotid sinus pressure.



A B C D

E

FIG. 10. (A–E)

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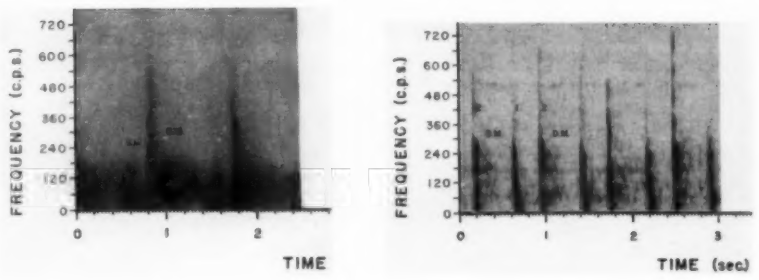


A B

FIG. 11. Aortic regurgitation. A. Advanced. B. Early.

is indicated by the high frequencies attained, the presence of harmonics and the increase in over-all intensity. Systole is relatively clear at the apex in the case of clinically "pure" mitral stenosis represented in A and B. There is splitting of the second heart sound. The opening mitral "snap" is responsible for one of the components demonstrated. The gap between the second sound proper and the beginning of the diastolic rumble is noteworthy and should be compared with the finding in aortic regurgitation (see Figure 11) and in mitral stenosis with much mitral regurgitation (see Figure 10E). This gap represents isometric relaxation. It appears that an opening mitral "snap" is demonstrated in A. It is located between the second sound and the beginning of the diastolic murmur. It is curious that in many cases it should be louder at the left sternal border and pulmonary area than in the mitral area. Such has been commented on before (15). The second component of the "split P2" in Figure 10C is apparently opening mitral "snap". In 10D there is no true opening "snap" although the abrupt onset of the diastolic murmur might impress one as an opening "snap".

With mitral regurgitation (see Figure 10E) the ringing quality of the first sound is less conspicuous and there is virtually no interruption between the systolic and diastolic murmurs. No opening "snap" is discernible.

Occasionally an early systolic click (17) was recorded at the pulmonary area in the cases of mitral stenosis. There may be a slight sound of this nature introducing the systolic murmur in Figure 10C.

In almost all instances the diastolic murmur is first decrescendo and, in those cases with sinus rhythm, crescendo in presystole. The decrescendo and crescendo characteristics apply not only to intensity but also to peak frequency. The diastolic murmur is obviously lower pitched than that of aortic regurgita-

FIG. 10. Mitral stenosis. A. Apex in clinically "pure" mitral stenosis with sinus rhythm. B. Same as A with logarithmic frequency scale. C. Pulmonary area corresponding to A. D. Mitral stenosis with atrial fibrillation. E. Mitral stenosis with considerable mitral regurgitation; atrial fibrillation.

tion; it is this which is responsible, at least in part, for its rumbling quality. The cases presented in D and E illustrate the absence of presystolic element when atrial fibrillation is present.

A glance at A, B and C will make clear the reason for the student's frequent confusion about what is systole and what diastole in such cases as this. Furthermore, the spectral phonocardiogram reproduces closely Duroziez's (22) valuable onomatopoetic mnemonic device, "fout-ta-ta-rou" (Fig. 10A).

AORTIC REGURGITATION

Figure 11A presents the recording in a case of aortic regurgitation due to rheumatic valvulitis. The recording was made in Erb's area (left sternal border). The diastolic murmur is striking and shows a decrescendo character both as to intensity (as indicated by the shading from black to gray) and as to peak frequency. Figure 11B presents a softer aortic diastolic murmer.

Figure 12 presents graphically the differences resulting from use of different types of chest pieces. The diaphragm greatly attenuates the lower frequency components and accentuates those of higher frequency. One would suspect that the natural frequency of the diaphragm employed is approximately 300 cycles.

MISCELLANEOUS SONIC PHENOMENA OF CARDIOVASCULAR ORIGIN

In Figure 13 is demonstrated the sound spectrogram of a *uterine souffle* recorded from the anterior abdominal wall of a pregnant woman. This is a continuous murmur with systolic accentuations. There is a striking similarity to an arterial pulse pressure curve. The resemblance is probably more than coincidence. The frequency variations observed here are clearly related to

FIG. 12. The same murmurs recorded from a bell and from a diaphragm chest piece with all other conditions of recording and analyzing identical.

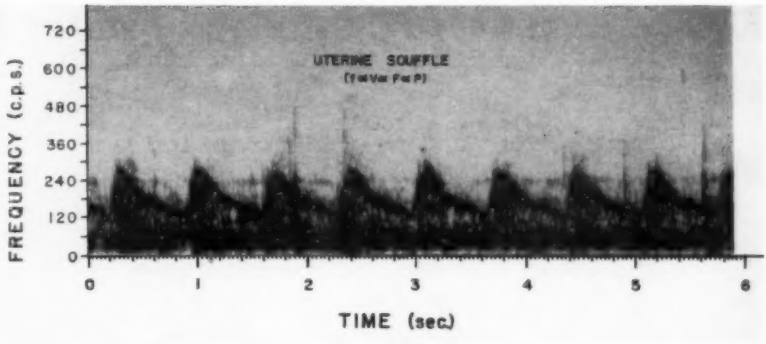


FIG. 13. Uterine souffle. (Sounds due to fetal movements are seen at three points.)

variations in the velocity of blood through the generator area. This in turn is directly related to variations in flow. Flow in turn, assuming substantially constant resistance through one cardiac cycle, is directly proportional to pressure.

Three examples of *cardiorespiratory murmurs* have been studied. In the case of two of them (examples are shown in Figure 14) the adventitious sound was in early diastole. In the third case it was in late systole. All were present predominantly in the latter part of inspiration. The spectral phonocardiogram appears to afford certain differentiation from a murmur of intracardiac origin. This was a point of importance in all three patients since they all had rheumatic heart disease or had had rheumatic fever. The early diastolic cardiorespiratory sound was easily taken for the murmur of aortic regurgitation. The characteristics of the cardiorespiratory murmurs are as follows: 1) a constant position in the respiratory cycle, on the one hand, and in the cardiac cycle on the other; 2) a fundamental with a characteristically sloping frequency-time configuration; 3) constant pitch, although the intensity shows marked variations to the point of extinction in some phases of respiration. The loudest of the three cardiorespiratory murmurs could be demonstrated to have harmonics (see Figure 14A) and it seems likely that most do. Of some interest is a consideration of the factors in the genesis of these murmurs. One patient (V. W., 106004) with the loudest C-R murmur, which was in systole, had moderate cardiomegaly making compression of a lappet of lung a plausible mechanism. Both she and a second patient had had rheumatic fever with possible pericardial involvement. The third patient demonstrated the cardiorespiratory murmur two weeks following mitral valvulotomy. A pericardial or pleuropericardial origin of the two cases of early diastolic C-R murmur seems likely.

In Figure 15 is presented the recording of a six year old girl (M. M., 601659) with *inter-atrial septal defect.* As is shown in this recording from the apical

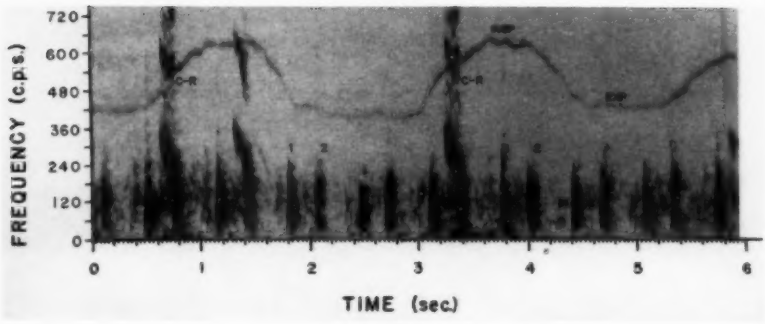


FIG. 14A

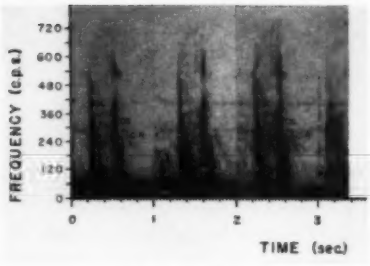


FIG. 14B

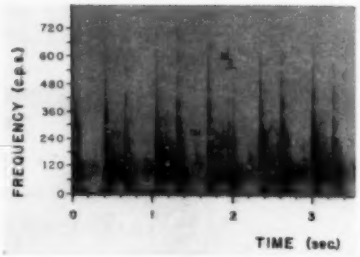


FIG. 15

FIG. 14. Cardiorespiratory murmurs. A. In late systole. (Note "scratchy" artefact in left lower corner.) B. In early diastole. ("C-R" indicates murmur; S.C., the early systolic click; O.S., the opening mitral snap.)

FIG. 15. A mid-diastolic murmur at the apex with inter-atrial septal defect. Bundle branch block.

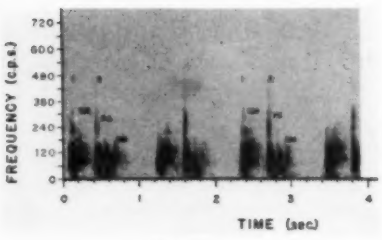


FIG. 16

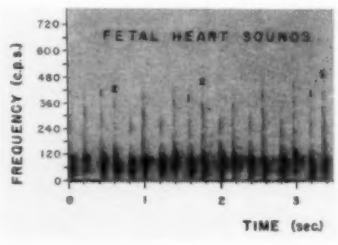


FIG. 17

FIG. 16. Protodiastolic sound and diastolic murmur at apex one year after decortication of heart for constrictive pericarditis.

FIG. 17. Fetal heart sounds.

area there was splitting of the heart sounds, particularly the second, and a mid-diastolic sound. The splitting of the heart sounds was the result of right bundle branch block (see Figure 4 for a similar case) and the mid-diastolic sound is that frequently encountered in patients with this variety of cardiac malformation (3) (12). There was no evidence of mitral stenosis at operation for closure of the septal defect. The possibility of the mistaken diagnosis of mitral stenosis is great in such cases.

Figure 16 presents a puzzling *diastolic sound* (following a protodiastolic sound) heard for the first time about one year after the patient (E. S., 372347) had had decortication for constrictive pericarditis which was presumably tuberculous in etiology. The patient was clinically well. Luisada and Montez (3) described a similar sound in a patient who had adhesive pericarditis but apparently did not have Pick's syndrome. In the specific patient whose murmur is illustrated in Figure 16 there was evidence he had persisting ventricular dilatation as sequel to the disuse atrophy of the myocardium which developed during his prolonged period of cardiac compression. The protodiastolic sound may be a gallop and the murmur due to relative mitral stenosis. An adventitious protodiastolic sound is frequently heard in untreated constrictive pericarditis (11).

Fetal heart sounds are displayed in Figure 17. It is of interest that in spite of the very thick abdominal wall peak frequencies of about 500 cycles are demonstrated.

In Figure 18 the effect of an *extrasystole* on the murmurs of aortic stenosis and regurgitation is demonstrated. Note that after the compensatory pause the systolic murmur is louder and has higher frequency components.

In Figure 19 the auscultatory findings (at the pulmonic area) of *patent ductus arteriosus* are demonstrated. The murmur tends to surround the second heart sound which is accentuated. Occasionally we have recorded an early systolic sound in the pulmonic area of these patients and in others with dilated

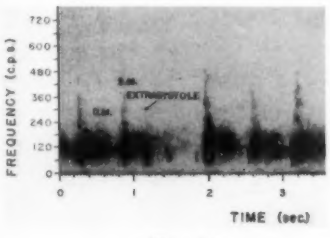


FIG. 18

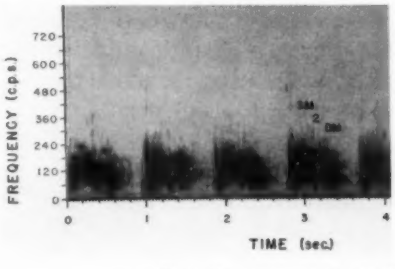


FIG. 19

FIG. 18. Effect of extrasystole on the murmurs of aortic stenosis and regurgitation. (Note conspicuous harmonics in last systolic murmur.)

FIG. 19. Patent ductus arteriosus (pulmonary area). "S.C." refers to systolic click.

pulmonary artery (17). Note that the build-up of the continuous murmur around P2 extends both to intensity and to peak frequency.

The *pistol-shot* sound (not illustrated) over peripheral arteries in cases of advanced aortic regurgitation is a sharp, short sound and has easily discernible harmonics with high peak frequency (about 2,000 cycles).

THE PHYSICAL NATURE OF VARIATIONS IN THE QUALITY (TIMBRE) OF HEART SOUNDS AND MURMURS

Quality of heart sounds and murmurs is a feature which has eluded exact description in the past. It is one of the most difficult aspects of clinical auscultation to transmit to the student. A large number of adjectives and other terms have been introduced to describe quality. A few of these are the following: shrill, musical, rasping, ringing, booming, rumbling, tambour-like, cooing, snapping, crescendo, decrescendo. At times in the past too much diagnostic significance has been attached to variations in quality. Gee is said to have referred to these as "vain distinctions". However, inasmuch as these variations must have a basis in anatomical or hemodynamic variations in the generator or in the surrounding tissues, definition in physical terms of various qualities of heart sounds and murmurs should not be entirely without significance. It might be possible to attach more significance to variations in quality than is possible in our present state of ignorance of basic mechanisms in connection with heart sounds.

Since spectral phonocardiograms are total descriptions of heart sounds and murmurs, quality is displayed. This point is dramatically illustrated by the spectral phonocardiograms of the type of aortic diastolic murmur variously described by analogy to the cooing of a dove, the cry of a sea-gull, wood sawing, the humming of a top, etc. Eversion of an aortic cusp on the basis of syphilis is most frequently the cause (6) and was the basis in the two cases in whom we have recorded spectral phonocardiograms. The first of these was illustrated in a previous publication (1). Figure 20 illustrates the second case. At the base the diastolic murmur was so loud, being audible at a distance from the chest, that by comparison the normal first heart sound is too faint to be demonstrated. In this case it is again seen that the characteristics of this type of murmur are 1) the presence of clearly defined fundamental and harmonics and 2) in these a curvature which is responsible for the "o͡o" of the "coo". Figure 20 A shows one at the moment unexplained feature, namely the interruption in the third and fourth harmonics. Figure 20 B illustrates the alteration in the murmur which occurs in transmission to the apical area. The fundamental and second harmonic—those components which are in the general range of the natural frequency of the thorax—are best transmitted. This resulted in the expected change in stethoscopic character of the murmur as heard at the apex. (It is

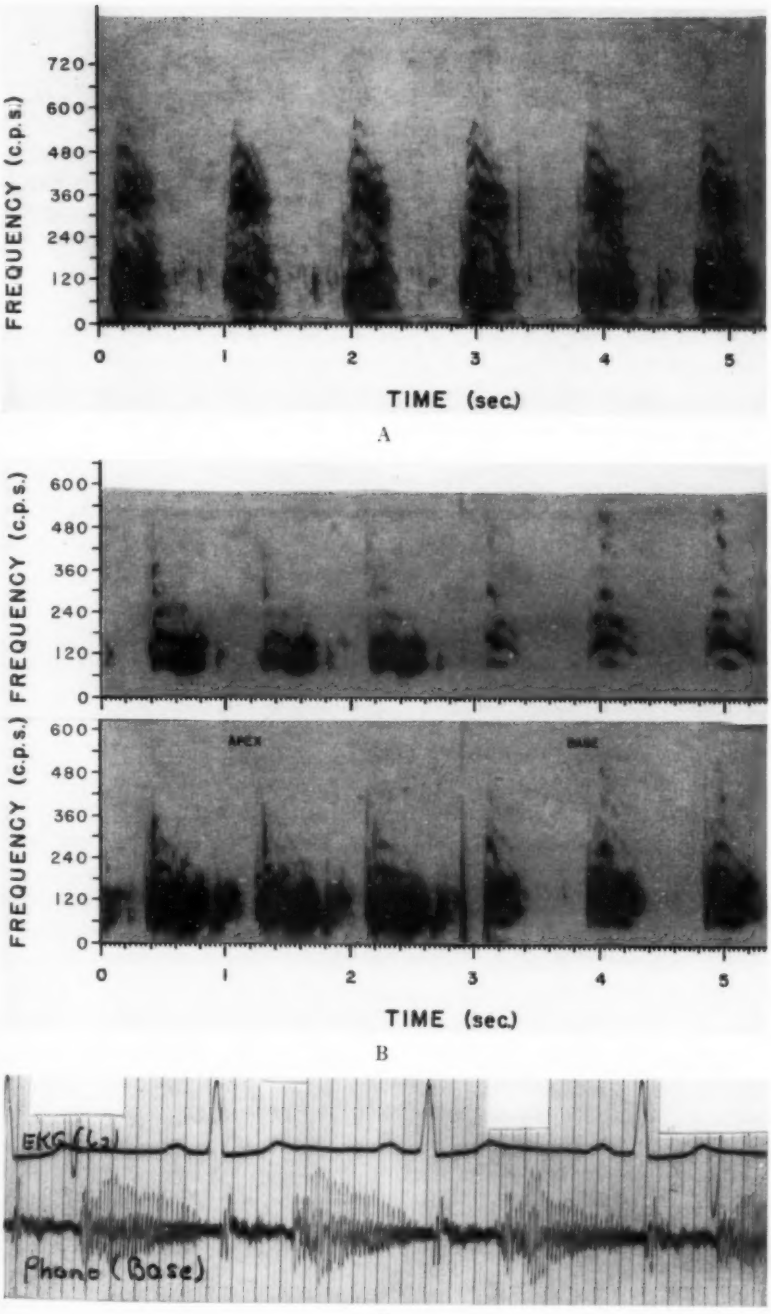


FIG. 20. A sea-gull aortic diastolic murmur. A. At the base. B. A comparison of the murmur at the base with that at the apex. (Two amplifications, above and below.) C. Same murmur presented as conventional phonocardiogram.

to be noted that the murmur is longer at the apex and that the curvature in the fundamental and harmonics is less conspicuous.)

Figure 20C presents a conventional phonocardiogram recorded at the base in the same patient. On close inspection the second and third harmonics can be seen superimposed on the fundamental. (Wells and his collaborators (9) presented a conventional phonocardiogram of a sea-gull diastolic murmur in a rheumatic patient. Here too, although the authors did not comment on this feature, fundamental and harmonics can be made out. Other authors have published phonocardiograms of musical murmurs with only the fundamental being registered. It is likely that the instruments used were not sufficiently sensitive in the range of the harmonics of higher frequency.)

Ringing sounds usually have conspicuous harmonic components. This is illustrated by the ringing first sound at the apex in mitral stenosis (see Figure 10).

Systolic murmurs, of aortic stenosis for example, which to the ear are rasping, musical, or shrill, usually show harmonic components. This fact is illustrated by the last systolic murmur in the record of Figure 18.

INTENSITY-FREQUENCY RELATIONSHIPS

It is clear that in general intensity and peak frequency go hand in hand. The louder a sound or murmur or a portion of a sound or murmur, the higher the peak frequency content demonstrated by such methods as the one under discussion. For example the murmur of aortic stenosis, described as diamond-shaped (13) because of its intensity peak in mid-systole as displayed by classical phonocardiograms, also have a frequency peak in mid-systole (see Figure 18; also Figure 4C and D in ref. 1). Similarly murmurs which are described as crescendo or decrescendo on the basis of intensity-time characteristics are also crescendo or decrescendo in character as far as peak frequency is concerned. See Figure 11 for illustration of this point in the decrescendo diastolic murmur of aortic regurgitation and Figure 10 for the decrescendo and crescendo characteristics of the diastolic murmur of mitral stenosis.

Because of the close dependence of frequency pattern on intensity, studies of frequency content in normal and abnormal cardiovascular sound, recorded either at the surface of the chest or from the surface of the heart and great vessels, must provide for careful standardization and calibration of the intensity dimension. The conclusions arrived at by studies of intrathoracic and direct cardiac recordings are of doubtful value if such calibrations were not made (21).

In most situations the greater the amplification applied to a sound the lower (within limits) is the impression of average pitch appreciated by the listener. This is a result of the Fletcher-Munson characteristics of the hearing mechanism (14). The same must be true to some extent in listening to the

heart either directly with the stethoscope or by some indirect means. However, in the case of cardiac auscultation, an opposite cancelling action is present, namely the appearance, or rather the sensing, of higher frequency elements as intensity is increased.

In general no exception can be taken to the statements of Cabot and others (7) (8), made thirty or more years ago, that *when picked up at the surface of the chest* the overwhelming portion of the energy content of all heart sounds and murmurs will lie in a frequency range below 660 cycles. The murmur of aortic stenosis has in some instances shown weak components to 1400 cycles. Furthermore occasionally much accentuated sounds have frequency components in excess of 3000 cycles (see Figure 23B). As for the normal heart sounds proper, of adult subjects, rarely are there significant frequency components in excess of 200 cycles.

In relation to intensity-frequency considerations it should be noted that the low-frequency compression employed in these studies is necessitated by the great dynamic range of the heart sounds. Because of the limits of the two magnetic recording systems, some attenuation of the intense lower frequency components must be imposed in order to display at all the higher pitched (and fainter) components. The ear is more sensitive to the upper portion of the frequency range of heart sound and murmurs. In order to end up with displays which can match the ear in the higher frequency range it is necessary to employ low frequency compression. This is a problem well-recognized in conventional phonocardiography and equally pertinent here.

THREE DIMENSIONAL MODELS

As was discussed in the preliminary publication (1) "sections" (frequency—intensity analyses) can be made at specific points in time. When these are "stacked" a three dimensional model results. Such a model is shown in Figure 21. Their construction is laborious and they do not contribute enough to the understanding of these sounds to make them worthwhile except as teaching

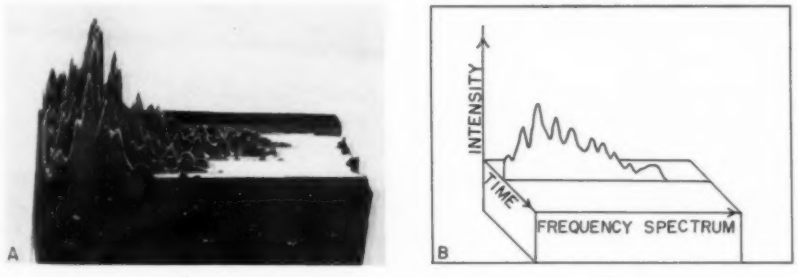


FIG. 21. A three dimensional model of the "sea-gull" aortic diastolic murmur shown in Figure 20. The harmonics and their curvature are demonstrated.

demonstrations of some classical murmurs. Even here the two dimensional display with the third dimension represented as degree of blackness performs a comparable function.

ARTEFACTS

In spectral phonocardiography artefacts are of four general types: first, those due to extraneous physiologic noise such as borborygmi and that associated with respiration; secondly, those due to ambient vibrations; thirdly those due to electronic noise of the recording and analyzing equipment; and finally, distortions introduced inadvertently by the amplifiers, the two magnetic recording steps, and the graphic display unit.

Artefacts due to ambient noise or to non-cardiovascular body noise can usually be recognized during editing of the tape recordings. Furthermore their characteristics in the spectral phonocardiogram have, for the most part, been recognized. In Figure 22 is an example of borborygmi as well as 240 cycle electrical interference. Spectral phonocardiography shares with the ear the advantage of being able to recognize many of these artefacts and therefore disregard them.

Inadvertent distortions and artefacts from the recording and analyzing equipment must be constantly guarded against. Change in the speed of the kymograph drum on the analyzer from that at which frequency calibrations were performed will alter the apparent frequency scale. Irregularity in the rotation of the kymograph drum will produce "wow" particularly in the harmonics of higher frequency. It is more striking in the upper frequency range since the percentile change in speed of the drum causes an identical percentile change in frequency of the sound being analyzed. This artefact can be especially troublesome in connection with the EKG and similar timing events when these

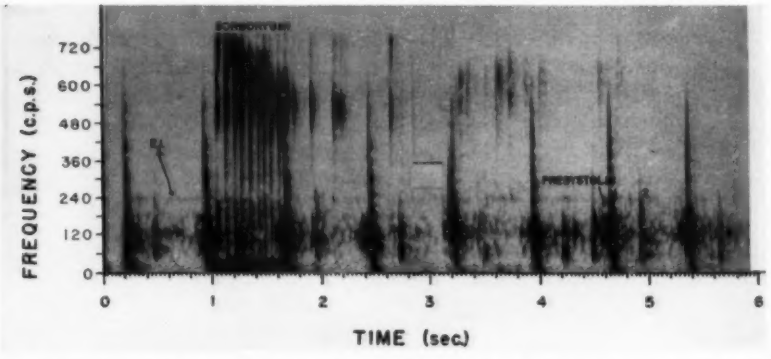


FIG. 22. Some artefacts—borborygmi and 240 cycle electrical interference (E.I.). Mitral stenosis with presystolic murmur and accentuated first sound.

are recorded by means of high frequency carriers. With a logarithmic frequency scale this type of artefact is less apparent.

Technical inexpertness in the analyzing may result in imperfections in the records such as "scratching" (see Figure 14A for example) which is due to poor marker-drum contact either because of a dirty drum or loose application of the paper.

PEDAGOGIC APPLICATIONS

Spectral phonocardiograms as now recorded resemble closely the mental image of heart sounds and murmurs. Experienced stethoscopists have little difficulty in identifying "unknowns" even without the parallel recording of a reference event such as the EKG. Persons indoctrinated in classical oscillographic phonocardiography sometimes have difficulty becoming accustomed to the fact that frequency spectrum, rather than intensity *per se*, is displayed on the ordinate. For instance, a higher peak tends to impress them as a louder sound. Since over-all intensity and peak frequency parallel each other closely, the confusion is not great.

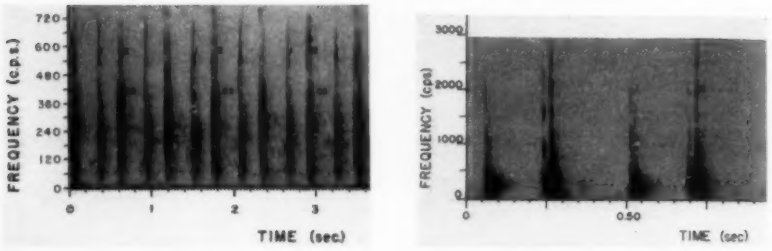
For students who are having their first introduction to clinical auscultation, spectral phonocardiograms appear to be a great boon. In the last year we have used them to a considerable extent in the teaching of physical signs, both cardiac and pulmonary. Although it is practically impossible to quantitate these observations, it is our impression that learning auscultation was both faster and more thorough when spectral phonocardiograms were displayed as the student listened to the patient's heart with his own stethoscope or to a magnetic tape recording of the heart sounds.

APPLICATIONS IN CLINICAL RECORDING

Inasmuch as the three dimensions of sounds are incorporated in a single, detailed two-dimensional display, spectral phonocardiograms are valuable in clinical practice for recording the course of certain forms of cardiovascular disease and for documenting certain features of the effects of cardiovascular surgery. Calibration and standardization is of importance particularly in connection with this application. These displays are ideal for detailed representation of cardiovascular sound on the printed page.

FURTHER AREAS FOR CLINICAL INVESTIGATION; FLEXIBILITY OF SPECTRAL PHONOCARDIOGRAPHY

Since it provides total analyses, which are susceptible to quantification, spectral phonocardiography is an ideal method for studying clinical and physiological problems concerning cardiovascular sound. The initial recording on magnetic tape with subsequent analysis of selected segments increases the flexibility of the method. The operation of the thorax on sound generated in



A B

FIG. 23. A. Pulmonic area in clinically "pure" mitral stenosis with subtile Graham Steell murmur. B. Same with the time scale "stretched" about four times.

the cardiovascular system, of the stethoscope on sound reaching the surface of the chest, of hemodynamic and anatomical factors on the several parameters of these sounds is amenable to study using this analytical tool.

As pointed out by Orias and collaborators (2) (4) (see critique of Mannheimer (19)) and most recently by Rodbard (10) one can, by spreading out the sounds in time and focusing attention on the upper part of the frequency spectrum, distinguish separate elements in the heart sounds proper. (In the first sound one might, for instance, be able to distinguish four constituents representing, respectively, mitral closure, tricuspid closure, aortic opening, and pulmonic opening.) The higher energy content in the lower frequency range makes for merging of these constituents whereas with the lower intensities present at the frequency peaks the constituents tend to be distinct.

This spreading out of the heart sounds can be done in conjunction with spectral phonocardiography. An example is shown in Figure 23 A and B. The recording was made from the pulmonary area in a patient with clinically "pure" mitral stenosis. A subtile Graham Steell murmur is present. When the heart sounds are spread out four times (see Figure 23B) there are seen to be at least two distinct constituents of the first sound and three of the second sound. One must then resort to physiological correlations in order to identify the elements so dissected out. In the second sound of this case it is almost certainly the closing sound of the pulmonary valve which is so greatly accentuated both as to intensity and as to peak frequency content.

Sound spectrography can be applied with advantage to the study of normal and abnormal respiratory sounds. Analyses of percussion notes, egophony, "E to A change", rales of various types, normal and altered breath sounds, etc., will be presented in a separate communication, together with a discussion of the physical basis of these signs.

SUMMARY AND CONCLUSIONS

1. Spectral phonocardiograms of a variety of heart sounds and murmurs have been presented and discussed.

2. Technical modifications which permit simultaneous display of EKG, respirations, and other physiologic phenomena and those necessary for a logarithmic frequency scale are described briefly and examples of recordings presented.

3. Since all features of the sounds are visually displayed, quality (timbre), which previously has eluded exact description, can now be defined in physical terms. The "sea-gull" diastolic murmur and terms such as ringing, rasping, rumbling, etc. are so defined.

4. Preliminary observations are presented concerning the operation of one part of the stethoscope on sound reaching the surface of the chest (Fig. 12), the operation of the thorax on sound generated in cardiovascular structures (Fig. 20 [illegible]), and the dissection of heart sounds by this method (Fig. 23).

5. Crescendo, decrescendo, diamond-shaped and similar terms derived from the intensity characteristics as displayed by classical phonocardiography are found to apply to frequency characteristics as well. In general, peak frequency and over-all intensity vary in a parallel fashion in sounds of cardiovascular origin.

6. In the case of one type of vascular sound (uterine souffle) the relation to the generating system and the operation of hemodynamic factors on this sound are discussed. The similarity of the uterine souffle to an arterial pulse pressure curve is pointed out (Fig. 13).

7. Spectral phonocardiograms resemble closely the mental image of heart sounds and murmurs.

8. Spectral phonocardiograms facilitate the learning of clinical auscultation.

9. Inasmusch as all three dimensions of sound are incorporated in a single detailed display, spectral phonocardiograms are valuable for permanent clinical records and for the detailed presentation of these sounds on the printed page.

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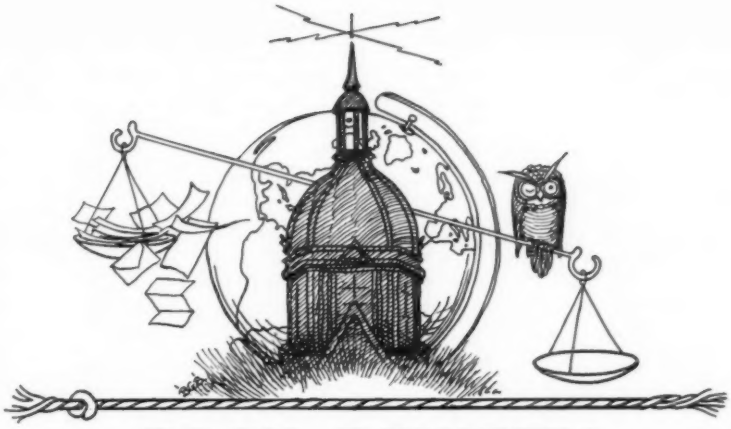

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of members of the Editorial Board of this Bulletin)

Einfuhrung in Die Hals-Nasen-Ohrenheilkunde, (Introduction to Otolaryngology) 2nd edition. By PAUL FALK. 175 pp., Ganzleinen DM 19.80. Georg Thieme Verlag, Stuttgart, Germany.

This book presents in very compact form an abundance of information on the anatomy, physiology and special methods of examination in otorhinolaryngology. There are adequate embryological descriptions. Special reference is made to the topography of the different regions. Audiometry receives only short mention. The material is well organized and very readable. Print and illustrations are excellent. While this book is written primarily as an introduction to otorhinolaryngology it will be of value also to the specialist for quick reference.

WALTER E. LOCH

Geschwülste des Ohres und des Kleinhirnbrückenwinkels. By K. GRAF. 283 pp., Ganzleinen DM 43. Georg Thieme Verlag, Stuttgart, Germany.

This book is an extensive analysis of benign and malignant tumors of the ear and the cerebello-pontine angle.

The major part of the book deals with acoustic tumors. Other chapters discuss neurinomas of the trigeminal, facial and glossopharyngeal nerves, meningeomas of the cerebello-pontine angle, glomus pigulare tumors of the tympanic cavity, carcinomas of the epipharynx invading the temporal bone, metastatic carcinomas of the temporal bone, sarcomas of the temporal bone, primary cholesteatomas and circumscribed arachnoiditis of the cerebello-pontine angle.

The author presents a thorough discussion of the clinical and pathological findings in 67 of his own cases and makes extensive reference to the literature. Of particular interest are the detailed microscopic descriptions and the x-ray findings.

This book can be highly recommended and should be of special interest to otologists as well as neurologists and neurosurgeons.

WALTER E. LOCH

Problems of Consciousness. Transactions of the Third Conference, March 10 and 11, 1952. Edited by HAROLD A. ABRAMSON. 156 pp., $3.25. The Josiah Macy, Jr. Foundation, New York.

The Third Conference Report contains three papers dealing with experimental approaches to the understanding of the nature of consciousness and the accompanying discussion recorded during and after the presentations. The participating group consisted of scientists in the fields of psychiatry, medicine, anthropology and neurophysiology. Their spontaneous discussion was the high point of this Josiah Macy, Jr. Foundation conference.

The papers included "Consciousness and the Metabolism of the Brain" by Seymour S. Kety, comparing states of consciousness including sleep, to various measurable psychological indices. The presentation of the paper was sidetracked for a time by a splendid discussion of Dr. Kety's diagram illustrating his mechanistic concept of human behavior. The second paper was "Hypnotic Phenomena" by Lewis R. Wolberg. Some of Dr. Wolberg's hypnotherapeutic techniques were described and illustrated and a portion of Lester Beck's movie illustrating the effects of repression on consciousness was presented to the group. The third paper was "Experimental Work on Sleep and Other Variations of Consciousness" by Marcel Monnier of the University of Geneva. Dr. Monnier reviews lucidly the neurophysiological correlates of consciousness in anatomical and electrical systems, including the electroencephalogram. Especially interesting to clinicians were the comparisons of alterations of consciousness produced by drugs to those produced by other natural and experimental events, including the effect of anxiety. The emphasis on the difficulty of measuring slight variations in consciousness such as those producable by anxiety was emphasized clearly. As an up-to-date review for anyone interested in human behavior, this book offers an interesting, illuminating and entertaining evening's reading. There is a good bibliography following each article.

ALLAN R. MCCLARY

General Biochemistry. By WILLIAM H. PETERSON AND F. M. STRONG. 469 pp., $8.65. Prentice-Hall, Inc., New York.

This is a brief descriptive survey of the major fields of biochemistry. There is more than usual emphasis on the biochemistry of plants and microorganisms, and on vitamins, nutrition and nitrogen fixation, consonant with the authors' relationship to the University of Wisconsin. There is inadequate coverage of topics such as acid-base balance, respiration and intermediary metabolism essential to a medical curriculum. The text would probably be suitable for an introductory course in an agricultural college.

F. P. CHINARD

Conferences on Drug Addiction Among Adolescents. Sponsored by the Committee on Public Health Relations of the New York Academy of Medicine with the Assistance of The Josiah Macy, Jr. Foundation. Conferences held at the New York Academy of Medicine, November 30, 1951 and March 13 and 14, 1952. 320 pp., $4.00. The Blakiston Co., New York.

Drug addiction among adolescents was the subject matter of two conferences under the auspices of the New York Academy of Medicine in 1951 and 1952. The discussions of the 52 contributors who represented the legal, educational, social, medical and law enforcement fields were recorded verbatim and published as such. In spite of this, the book is very readable and a source of a great variety of interesting information on addiction. The different addictive drugs and their physiological and psychological effects are presented. Some misconceptions, like the addictive qualities of marihuana, are dispelled. Community responsi-

bilities and lack of agreement on their implementation are highlighted. The unsatisfactory state of treatment for addiction is the subject of several papers which also stress the need for more research and public responsibility for this. An interesting glossary of drug addiction terms is appended.

L. H. GLIEDMAN

Side Effects of Drugs. By L. MEYLER. Tr. by Ph. Vuijsje and W. Mulhall Corbet. 268 pp., $5.50. Elsevier Publishing Co., Houston, Texas.

The aim of this book is an admirable one. It is intended, by pointing out the untoward effects which may follow administration of various drugs, to increase awareness of the occurrence of such complications, and to encourage the use of drugs only in situations in which they are specifically needed. The author expresses the hope that such practice would reduce the incidence of undesirable reactions to therapeutic agents, the number of subjects who become sensitive to these agents, and the occurrence of bacterial resistance to anti-bacterial agents. The author has compiled what is essentially a listing of the untoward effects that may be produced by therapeutic agents. The sections on heavy metals, sulfonamides, antibiotics, antimalarial drugs, hormones, antithyroid drugs, and anticoagulants are the most complete. While most of the information is sound, it is, in general, presented poorly. The untoward effects produced by each drug are listed, usually in abbreviated compendium form. In many instances differentiation is not made between those effects which are the pharmacologic effects of excessive doses, those which are due to an unusual reaction to ordinary doses, and those which are due to the development of hypersensitivity. The incidence and importance of the untoward effects, and the dose-effect relationships, are seldom noted. Very little information is provided concerning mechanism of drug action or of "side effects". The use of the words "side effects" is itself unfortunate, since in many instances they represent the pharmacologic effects of doses higher than the ordinary therapeutic range. It is also to be regretted that the treatment of untoward effects is presented for only part of the drugs listed. Where presented, the details of management are in general up-to-date, though there are a number of surprising recommendations, such as the administration of Vitamin B_1 in the treatment of untoward effects due to morphine, the treatment of hypersensitivity reactions to procaine with neostigmine, and the administration of 1 mg. of vitamin K with every gram of salicylate. The translation from the Dutch is stilted, being almost literal in places, but there is a fairly good supporting bibliography, two-thirds of which consists of English language references.

While the aim of this book is commendable, and while it may serve some use as a ready reference manual, it is to be regretted that the author did not present his material in a more systematic and useful manner.

DAVID GROB

BOOKS RECEIVED FOR REVIEW

Alcoholism. By JACKSON A. SMITH. 72 pp., $3.00. J. B. Lippincott Co., Philadelphia, Pa.

Annual Review of Medicine, Vol. V. WINDSOR C. CUTTING, Editor. 490 pp., $7.00. Annual Reviews, Inc., Stanford, California.

The Atom Story. By J. G. FEINBERG. Foreword by Prof. F. Soddy. 243 pp., $4.75. Philosophical Library, New York.

Behandlung Innerer Krankheiten. Richtlinien und Ratschläge fur Studierende und Ärzte.

By FERDINAND HOFF. 608 pp., Ganzleinen DM 55. ($13.10). Georg Thieme Verlag, Stuttgart, Germany.

Clinical Orthopaedics, No. 3. Edited by ANTHONY F. DEPALMA. 230 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

Der Herzkatheterismus bei Angeborenen und Erworbenen Herzfehlern. By OTTO BAYER, FR. LOOGEN, AND H. HELMUTH WOLTER. 191 pp., Ganzleinen DM 36. ($8.60). Georg Thieme Verlag, Stuttgart, Germany.

Fifty Years of Medicine. (An expanded version of the Harben Lectures delivered at the Royal Institute of Public Health and Hygiene, December 1952). By LORD HORDER. 70 pp., $2.50. Philosophical Library, New York.

Fundamentals of Otolaryngology, 2nd edition. By LAWRENCE R. BOIES. 487 pp., $7.00. W. B. Saunders Co., Philadelphia, Pa.

Histopathology of the Skin, 2nd edition. By WALTER F. LEVER. 518 pp., $12.00. J. B. Lippincott Co., Philadelphia, Pa.

Low Back Pain and Sciatica. By LOUIS T. PALUMBO. 104 pp., $3.00. J. B. Lippincott Co., Philadelphia, Pa.

A Manual of Tropical Medicine, 2nd edition. By THOMAS T. MACKIE, GEORGE W. HUNTER, III, AND C. BROOKE WORTH. 907 pp., $12.00. W. B. Saunders Co., Philadelphia, Pa.

Parkinsonism and Its Treatment. Edited by LEWIS J. DOSHAY. 152 pp., $3.00. J. B. Lippincott Co., Philadelphia, Pa.

Proceedings of the Forum Sessions; Thirty-Ninth Clinical Congress of the American College of Surgeons, Chicago, October, 1953. I. S. RAVDIN, Chairman. 752 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa.

Psychology, the Nurse and the Patient. By DORIS M. ODLUM. 168 pp., $4.75. Philosophical Library, New York.

Textbook of the Nervous System, 2nd edition. By H. CHANDLER ELLIOTT. 437 pp., $9.00. J. B. Lippincott Co., Philadelphia, Pa.

Veterans Administration Technical Bulletins, Series 10, Vol. VI, 1952 and 1953. Edited by ARTHUR M. WALKER. 44 pp. (Not for sale; a few presentation copies are still available for medical libraries upon request.)

Thomas Young. Natural Philosopher 1773–1829. By the late ALEX WOOD and completed by FRANK OLDHAM with a memoir of Alex Wood by C. E. RAVEN. 355 pp., $6.00. Cambridge University Press, New York.